A Review of Antifungal Activity of Combined Plant Extracts or Plant Exudates from Medicinal Plants either together or with Known Antifungal Agents

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ABSTRACT

Medicinal plants provide humanity with important phytochemical compounds and extracts which are widely used in treatment of many diseases. Fungal infections are one of these diseases which are widely distributed especially in developing countries; medicinal plants are extensively used in developing countries. There are few antifungal agents, most of them are expensive and have many adverse effects, also there is high incidence of drug resistance among some available antifungal agents, hence for these mentioned reasons many people, especially in developing countries, use medicinal plants (either alone, combined together or combined with known antifungal drugs) in treatment of many fungal infections. This rise a new and important issue about plant(s) – plant(s) and plant(s) - drug interactions.

The aim of this review is to try to fill the gap in understanding the interactions of plant(s) - plant(s) and plant(s) – drug(s) combinations by providing an overview of some evidence-based researches done in this field, so our review highlights many interactions between medicinal plants constituents with current available antifungal agents, these interactions may be synergistic, additive, indifferent or antagonistic, so, if there is any antagonistic effect, we recommend to avoid using the combination which caused this effect. We collected a lot of studies which studied the interactions between plant(s) (including extracts, isolated active constituents, essential oils, plants latexes and...
Keywords: Antifungal activity; plant-plant combination; plant-drug combination; synergism.

1. INTRODUCTION

The relationship between man and plants started from the earliest history and was developed, so, the herbalist is one of the first professionals in the evolution of human cultures. Today, with the improvements in technology and isolation methods, the plant kingdom is regarded as a big warehouse which contains many chemical compounds, some provide novel structures (lead compounds) from which synthetic chemists may derive even more interesting compounds [1].

Over the last few decades, there has been an universal considerable interest in traditional and complementary medicine (TCM). The World Health Organization (WHO) stated the main role of herbal medicines in preventive, promotive and curative on healthcare system, especially in developing countries [2].

A survey done in 2007 demonstrated that about 15% of patients are taking herbal products in conjunction with conventional pharmacotherapy, among these patients, herbal-drug interactions were observed in about 40% of patients; but it is often difficult to establish exactly what is the causative agent of this herbal-drug interaction, especially if it occurs in patients receiving multiple drug therapies (polypharmacy). This is a substantial public health issue since many patients who are known to be using one or more prescription drugs are also taking supplements preparations, and unfortunately only about one third of these patients were reported to tell their physicians about their use of these products together [3].

“Generally, there is a wrong belief concept in the public population that herbal medicines are safe because they are natural. However, this is a hazardous over simplification. Many different interactions and side effects to herbs have been reported and recently reviewed” [4].

“If two or more phytochemical compounds and/or plant extracts were combined together, and this combinations lead to changes in the overall biological effects and/or the bioavailability of each component, this will result in herbal-herbal or herbal-drug interactions. These interactions may be synergy if the mixtures enhance (for example) the antioxidant status, anti-inflammation, anti-cancer and chemoprevention of several oxidative stress” [5]. “In some cases, however, the combination of these mixtures may cause lower biological effects if they are administered in inappropriate ratios” [6]. “Sometimes the interactions of these mixtures may enhance or reduce the bioavailability of active compounds, often depending on many factors, like facilitation or competition in biological receptors or cellular uptakes and transportations” [7]. “On other hand, different classification methods were used to describe these positive or negative mixture interactions, they are potentiation, addition, synergism, or antagonism. There is a confusion about the differences between potentiation and synergy. If one active mixture(s) was/were mixed with another inactive mixture(s), but both mixtures produced a greater effect than that of the active mixture(s), the effect is known as potentiation (i.e. the presence of inactive mixture(s) enhanced the potency of active mixture(s))” [8]. If both mixtures were individually active, they can produce an additive, synergistic or antagonistic effect if they were combined together” [9]. Synergy comes from the Greek word “synergos”, which means “working together”. According to McGraw–Hill Concise Dictionary of Modern Medicine, synergy or synergism is defined as “the cooperative interaction between two or more components of a system, such that the combined effect is greater than the sum of each part” [10]. “Broadly, synergy is defined as the interaction or cooperation of two or more substances, organizations or other agents to produce a combined effect greater than the sum of their separate portions” [11]. “It is well known that herbal or plant extracts consist of a complex mixtures of natural compounds, this complexity may serve as a valuable resource for network-based multi-target drug discovery due to its potential treatment effects by synergy, for example, polyphenols and terpenoids are two groups of natural compounds which are available in many extracts, the former possess a strong
binding ability to different molecular structures like proteins or glycoproteins, while the latter have a great affinity for cell membranes and therefore, a high potential to permeate through cell walls of the body” [12]. “In this regard, synergistic expects may be observed in the interaction between herbal products and conventional drugs or biochemical compounds” [10]. Additive interaction is used to describe an effect of two combined active mixtures which produced similar potency of individual active components in each mixture; while antagonistic interaction appears when the combined effect is less potency than the sum of individual components potency of the mixture [13].

“Some fungi organisms have a harmful influence on the health and livelihood of mankind. The diseases caused by fungi are termed mycoses, today fungal infections are among the most difficult diseases to manage” [14]. “Figures say that there are about 1.5–5 million fungal species on the planet, only several hundreds of them can harm and cause disease in humans, and very few are able to affect healthy people” [15]. “Important progress has been achieved in an understanding of fungal pathogenicity including the mechanisms of adherence to host tissues, penetration of tissues, multiplication within the host and the interaction of fungal cells with host’s effector cells. In addition to the high increase in fungal infections caused by opportunistic and pathogenic fungi in compromised patients (like HIV positive patients), which are mainly caused by Candida spp., Aspergillus spp., Cryptococcus neoformans, Histoplasma capsulatum and Coccidioides immitis, many fungi that occur as saprophytes in the environment and which had previously been considered to be nonpathogenic are now being encountered as causes of human infection” [16].

“Mammalian hosts may acquire fungal infections by three ways; firstly, they may be exposed to truly pathogenic organisms that normally occur as saprophytes in the environment; secondly, individuals who are immuno-suppressed may acquire a fungal infection following exposure to weakly pathogenic organisms that occur as saprophytes in the environment, such organisms are termed opportunistic pathogens, infections such as aspergillosis and candidosis are being seen increasingly in immuno-compromised patients, particularly those with haematological malignancies or the acquired immune deficiency syndrome (AIDS); thirdly, individuals may be exposed to infective propagules of the dermatophyte fungi, organisms which are very well adapted to parasitism and quite capable of invading the healthy host, occasionally, dermatophyte fungi are found on the skin and scalp of individuals in the absence of symptoms, this is thought to represent transient colonization or a carrier state” [16].

Hence fungal infection diseases are classified into a number of broad groups according to the initial site of infection, this brings out clearly the degree of parasitic adaptation of the different groups of fungi and the way in which the site affected is related to the route by which the fungus enters the host:

(i) The superficial mycoses: These are infections which are limited to the outermost layers of the skin, nails, hair and the mucous membranes.

(ii) The subcutaneous mycoses: These are infections which are involved in the dermis, subcutaneous tissues and adjacent bone.

(iii) The systematic mycoses: These are infections that usually originate in the lung, but may spread to many other organs by blood or lymph circulation [17].

In comparison with the clinical availability of antibacterial drugs, there are very few number of antifungal agents. There are four major families of antifungal compounds: the polyenes, the azoles, the allylamines and the echinocandins. In addition, there is a miscellaneous group of antifungal compounds, such as flucytosine and griseofulvin, which do not belong to one of the major families [17].

“The polyenes (e.g. amphotericin B) bind to ergosterol, the principle sterol component of the fungal cell membrane resulting in a loss of cell wall integrity. The azoles (e.g. fluconazole, itraconazole, voriconazole and posaconazole) inhibit enzymes involved in ergosterol synthesis. The echinocandins inhibit glucan synthesis, glucan is a long chain polymer responsible for fungal cell wall stability, it accounts for 30–60% of the cell wall mass in Candida, Aspergillus and Saccharomyces species. Importantly, human cells do not contain glucan, thus accounting for the low rate of human toxicity associated with this class of agents” [18].

“The epidemiological data suggest that the incidence and prevalence of serious mycoses continues to be a public health problem. The increased use of antifungal agents has resulted
in the development of resistance to these drugs. The spread of multidrug-resistant strains of fungus and the reduced number of drugs available make it necessary to discover new classes of anti-fungal from natural products including medicinal plants. Medicinal plants have also been reported in traditional systems of medicine in the treatment of both human and animal mycoses, and are considered to be a valuable source for the discovery of new antifungal drugs” [19].

Specific Objectives:

1- To review and collect all researches done on medicinal plants combined together as antifungal (plant(s)- plant(s) combination).

2- To review and collect all researches done on medicinal plants combined with known antifungal drugs (plant(s)- drug(s) combination, isolated phytochemical compound- drug(s) combination, essential oil- drug(s) combination and plant latex- drug(s) combination.

2. METHODOLOGY

The data collection on the topic was conducted comprehensively by using international reliable databases for medical searching; i.e. PubMed (the National Library of Medicine), MEDLINE, Science Direct, Scopus, Google Scholar, Research gate, Wiley online library and other reliable databases for medical journals articles were used. Searches were not limited to publishing time.

The databases were thoroughly searched for studies that met the inclusion criteria. Search results were assessed for relevance according to the title, abstract and sometimes full text review. All abstracts were reviewed in relation to the inclusion/ exclusion criteria. Unless the abstract clearly described one or more exclusion criteria, the full article was then reviewed to check if it still met the inclusion criteria.

Any study was eligible for inclusion if it practically examined the effect of the plant(s)- plant(s) or plant(s)- drug(s) combination in term of either synergism, addition or antagonism. For all combinations (except some combinations of essential oils and phytochemicals with synthetic drugs) only studies that clearly mentioned the plants parts, extraction solvent and comprehensive results were considered as relevant.

Finally, the collected articles were reviewed one by one and the relevant information was extracted, analyzed, summarized and presented with their references.

3. RESULTS

3.1 Plant(s)-Plant(s) Combination

The following table (Table 1) shows some studies which had tested a combination of two or more plant extracts together as antifungal agents, and showed if there is any synergism, indifferent, additive or antagonism effects.

The leaves and stems (aerial parts) of Zuccagnia punctata (Fabaceae), Tetraglochin andina (Rosaceae), Larrea cuneifolia, L. nitida and L. divaricate (Zygophyllaceae) were separately macerated in ethanol. The antifungal activity was individually assayed in vitro against six yeast strains, they were Saccharomyces cerevisiae, Candida albicans, C. glabrata, C. tropicalis, C. parapsilopsis and C. krusei, the organisms were obtained from vaginal exudates of infected patients. The MIC values were determined for each extract, either alone or in combination between them; the FIC index was also calculated. According to the results report, "the synergist effect was observed with the combination between Z. punctata / L. cuneifolia against C. albicans, C. glabrata, and S. cerevisiae;also between Z. punctata / L. nitida and Z. punctata / L. divaricate extracts against C. glabrata (FIC index=0.5). An additive effect was observed with the combination of Z. punctata / L. cuneifolia against C. tropicalis and Z. punctata / L. nitida against both C. albicans and C. tropicalis (FIC index > 0.5 in both cases). Some combinations revealed indifferent interaction, in which the FIC indices were varying from 1.0 to 2.5. The most less active mixture was T. andina / L. nitida, which showed indifferent effect against all yeasts (FIC between 1.1 and 2.2). The results indicate that combinations between Z. punctata and Larrea speciesare more effective as antifungal than between Larrea speciesand between Larrea or Z. punctata with T. andina. The best combination was between Z. punctata and L. cuneifolia since it showed a synergistic or additive effect against all tested strains, indicating that the interaction between chemical components contained in both plant species extracts is more effective as antifungal activity" [20].
Table 1. Some examples of two or more plant extracts combined together and the type of interactions which they cause

<table>
<thead>
<tr>
<th>Latin name of plant extracts used</th>
<th>Part of plant used</th>
<th>Fungal organism(s) used</th>
<th>Type of interaction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zuccagnia punctata with Larrea cuneifolia</td>
<td>Aerial parts ethanolic extract (both)</td>
<td>Candida albicans, C. glabrata and Saccharomyces cerevisiae</td>
<td>Synergism</td>
<td>[20]</td>
</tr>
<tr>
<td>Zuccagnia punctata with Larrea nitida</td>
<td>Aerial parts ethanolic extract (both)</td>
<td>Candida glabrata</td>
<td>Synergism</td>
<td>[20]</td>
</tr>
<tr>
<td>Zuccagnia punctata with Larrea divaricate</td>
<td>Aerial parts ethanolic extract (both)</td>
<td>Candida glabrata</td>
<td>Synergism</td>
<td>[20]</td>
</tr>
<tr>
<td>Annona senegalensis</td>
<td>Leaves ethanolic extract with twig aqueous extract</td>
<td>Candida albicans, C. parapsilosis, C. neoformans and C. krusei</td>
<td>Antagonism</td>
<td>[21]</td>
</tr>
<tr>
<td>Annona senegalensis</td>
<td>Stem aqueous extract with twig aqueous extract</td>
<td>Candida albicans, C. parapsilosis, C. neoformans and C. krusei</td>
<td>Antagonism</td>
<td>[21]</td>
</tr>
<tr>
<td>Annona senegalensis</td>
<td>Leaves ethanolic extract with bark aqueous extract</td>
<td>Candida albicans, C. parapsilosis, C. neoformans and C. krusei</td>
<td>Antagonism</td>
<td>[21]</td>
</tr>
<tr>
<td>Annona senegalensis</td>
<td>Stem aqueous extract with bark aqueous extract</td>
<td>Candida albicans, C. parapsilosis, C. neoformans and C. krusei</td>
<td>Antagonism</td>
<td>[21]</td>
</tr>
<tr>
<td>Zuccagnia punctata with Larrea nitida</td>
<td>Aerial parts dichloromethane extract</td>
<td>Candida albicans and C. glabrata</td>
<td>Synergism</td>
<td>[22]</td>
</tr>
<tr>
<td>Baccharis glutinosa with Jacquinia macrocarpa</td>
<td>Aerial parts ethyl acetate fraction of methanol extract with aerial parts n-butanol fraction of methanolic extract</td>
<td>Aspergillus flavus and Fusarium verticillioides</td>
<td>Synergism</td>
<td>[23]</td>
</tr>
<tr>
<td>Dissotis multiflora with Paullinia pinnata</td>
<td>Leaves alcoholic extract with leaves alcoholic extract</td>
<td>Candida krusei and C. albicans</td>
<td>Synergism</td>
<td>[24]</td>
</tr>
</tbody>
</table>
"The effect of combined aqueous, ethanolic and hydro-ethanolic extracts of different parts (stem and barks, twigs and leaves) of Annona senegalensis (Anonaceae) was assessed against some pathogenic yeasts. The MIC of all extracts on the yeasts were ranging from 0.156 to > 5 mg/ ml. Candida krusei was the most sensitive yeast while Candida parapsilosis and Cryptococcus neoformans were the most less sensitive. Amongst the promising extracts, the aqueous extracts of the stem (SIH0), twig (TWH20), and bark (BH20) as well as the ethanolic extract of the leaf (LEIOH) were the most active against all tested yeasts (Candida albicans, Candida parapsilosis, Candida krusei and Cryptococcus neoformans), with MIC values ranged between 0.312 mg/ml and 2.5 mg/ml. The active extracts which exerted broad spectrum antifungal activity were considered for combination studies. The FICI of the combinations of LEIOH/ TWH20, SIH0/ TWH20, LEIOH/ BH20 and SIH0/ BH20 were varied from 5.50 to 48.09 on the four tested yeast strains, exhibiting antagonistic interactions (i.e. FICI> 4); the combinations led to 2- 4 fold reduction of antifungal activity as compared to the MICs of individual extracts. Thus, application of such combinations from different parts of A. senegalensis in the treatment of mycoses caused by C. albicans, C. parapsilosis, C. krusei and C. neoformans should be avoided" [21].

"The aerial parts of Zuccagnia punctata (Fabaceae) and Larrea nitida (Zygophyllaceae) were extracted with dichloromethane (DCM), and their extracts were tested alone and in combination of different ratios against Candida albicans and C. glabrata. The results showed that three over four Z. punctuate / L. nitida fixed - ratio mixtures displayed synergistic interactions against C. albicans. The doses of the most synergistic mixture was 65.96 μg/ ml (ZpE 72%). On the other hand, one over four Z. punctuate / L. nitida fixed mixtures displayed synergistic interactions against C. glabrata. The doses of the most synergistic mixture was 168.23 μg/ ml (ZpE 27%; LnE = 73%). The study concluded that the mixture of these plants especially at fixed doses which are most synergistic are of great interest for the development of an antifungal agents" [22].

"The leaves of Cassia alata (Fabaceae) and Ocimum sanctum (Lamiaceae) were macerated separately in ethanol 95% and a stock of each extract was prepared in 5 % dimethyl sulfoxide (DMSO) in the final concentration of 2 mg/ml. The macrobroth- dilution technique was employed for the susceptibility testing against cryptococcosis. The individual results revealed that the ethanolic extract of O. sanctum was inactive against all the strains up to a concentration of 1,000 mg/ ml (MIC), while the MIC of ethanolic extract of C. alata ranged from 500– 1,000 mg/m at acidic pH, the 1,000 mg/ml concentration of the extract was found to be fungicidal in action. Furthermore, the activity of the extract was recorded to be thermostable. The combination of both extracts inhibited the growth of the organism at a concentration ranging from 62.5– 125 mg/ml. A 125 mg/ml concentration of both extracts combination was found to be fungicidal in action. The combination of extract was heat stable and active at acidic pH" [25].

The study was conducted to investigate the antifungal activity of the hydro-distilled essential oil of Foeniculum vulgare (Apiaceae) seeds, the alcoholic extract of Nigella sativa (Ranunculaceae) seeds and the aqueous extract of aerial part of Camellia sinensis (Theaceae), they were used alone and in combination against 39 different Candida species, they were C. albicans, C. tropicalis, C. krusei, C. glabrata, C. dubliniensis and other Candida sppisolated from denture wearers. Three different concentrations were prepared from the tested plants as follows:

(i) N. sativa (20 μL) + F. vulgare (5 μL) + C. sinensis (5 μL);
(ii) N. sativa (15 μL) + F. vulgare (10 μL) + C. sinensis (5 μL);
(iii) N. sativa (10 μL) + F. vulgare (15 μL) + C. sinensis (5 μL).

"The results was reported as zones of inhibition; all plant extracts showed remarkable antifungal activity except the aqueous extract of C. sinensis against almost all of the tested Candida strains. The best anti-candidalactivity was found with the alcoholic extract of N. sativa (mean value = 12.3 mm), followed by the essential oil of F. vulgare (mean value = 7.9 mm); the results also exhibited that all mixed herbal extracts ranging from 7.8 to 15 mm, 7.6 to 15.5 mm and 7 to 15 mm inhibition zones for number (i), (ii) and (iii), respectively. The highest inhibition zone was related to mixture number (ii) (mean value = 12.3 mm), followed by mixture number (i) (mean value = 12.1 mm) and finally mixture number (iii) (mean value = 10.8 mm). Although lower concentrations of N. sativa along with higher concentrations of F. vulgare led to lowest antifungal activity of herbal mixtures, but there were no significant differences in action between the three herbal
mixtures. The highest and lowest activities of the tested mixtures were seen against C. krusei and C. albicans respectively” [26].

Aerial parts of Baccharis glutinosa (Asteraceae) and Jacquinia macrocarpa (Primulaceae) were extracted with 70% methanol. The obtained extracts were sequentially fractionated with hexane, ethyl acetate and n-butanol after being suspended in water. The ethyl acetate fraction of B. glutinosa and the n-butanol fraction of J. macrocarpa were mixed together and were tested for antifungal activity against Aspergillus flavus and Fusarium verticillioides, because these fractions were individually active. The MIC$_{50}$ of each fraction was determined and the Fractional Inhibitory Concentration index (FIC index) was also calculated in order to evaluate their synergistic effect. The MIC$_{50}$ of ethyl acetate fraction of B. glutinosa against A. flavus was 1.1 mg/ ml, whereas the MIC$_{50}$ of n-butanol fraction of J. macrocarpa against F. verticillioides was 0.3 mg/ ml, the FIC indices of the combination against A. flavus and F. verticillioides were 0.5272 and 0.4577 respectively, indicating a synergistic effect against both species. The synergistic effect was even seen at lower concentrations than those of the individual fractions. Only 12% and 8% of A. flavus and F. verticillioides spores treated with the synergistic mixtures respectively, were able to germinate [23].

The leaves of Monodora tenuifolia (Annonaceae), Terminalia catappa and T. mantaly (Combretaceae) were extracted with distilled water, 70% and 95% ethanol respectively. The extracts were tested against Candida albicans, C. glabrata and C. parapsilosis by dilution method using Muller Hinton Agar. MIC and MFC were calculated. In individual assay, the results showed that extracts from Terminalia speciess were the most active with their MIC ranged between 0.0781 and 2.5 mg/ ml for T. catappa, and from 0.0391 to 0.3125 mg/ ml for T. mantaly; except for the ethanolic extract of T. catappa which is fungistatic on C. glabrata, the others extracts from this plant were fungicides on the tested yeasts. All the extracts of T. mantaly were fungicides on C. glabrata and C. albicans. Combinations of active sub-fractions were tested by checkerboard method with some modifications, FICI and building isobolograms methods were applied, it was concluded that there was no change in the activity of the sub-fractions from T. catappa in comparison with the partitionated fractions; with sub-fractions from T. mantaly, there were an increase in their activity compared to those of the previous fractions; the sub-fractions from M. tenuifolia were less active than their fractions [27].

Toddalia asiatica (Rutaceae) roots, Rhamnus staddo (Rhamnaceae) roots, Momordica foetida (Cucurbitaceae) shoots, Podocarpus falcatus (Podocarpaceae) bark, Aloe spp (Asphodelaceae) succulent leaves and other individual plants were tested alone and in combinations against Aspergillus niger and Candida albicans. Hot water, cold water and dichloromethane/ methanol (1:1) were the solvents used in their extraction. The aqueous extracts of these plants were inactive when tested alone, the DCM/ methanol extract of P. falcatus showed the highest activity (77.77% inhibition) against A. niger while DCM/ methanol extract of M. foetida showed the highest activity (77.78% inhibition) against C. albicans. Aloe spp. was inactive against A. niger. The combination of extracts (including the inactive Aloe spp. extract against A. niger) showed antifungal activity against C. albicans and A. niger; but the antifungal activity of all combinations was similar to the activities of all individual extracts against C. albicans. On the other hand, against A. niger, the individual extracts of T. asiatica and Aloe spp. were comparatively lower than that of their mixture. Hence a mixed actions of antagonism, additive and synergism were observed in this assay [28].

“The stem-bark of Euphorbia abyssinica (Euphorbiaceae) and the whole plant of Coleus species (Lamiaceae) were extracted with methanol. The plant extracts were both assayed individually and in combinations (using a pour-plate method) against Candida albicans, Trichophyton mentagrophytes, Microsporum gypseum and Epidermophyton floccosum. Two assay methods were employed, they were checker board assay and time kill assay. The most potent was Coleus spp. extract, as its effect on C. albicans and T. mentagrophytes cells showed that the MIC and at double the MIC concentrations decreased the cell counts to about 0.05 log 10 in 48 hours; this same double MIC (15.6 mg/ ml) killed M. gypseum cells in 6 hours only. However, when C. spp. and E. abyssinica extracts were combined, they exhibited no synergistic interactions against C. albicans, T. mentagrophytes and M. gypseum” [29]. “In 48 hours, Coleus spp. at MIC of 0.98 mg/ ml decreased E. floccosum viable cell counts from 1x10$^5$ CFU to 0.97 log10; when the MIC
was doubled to 1.96 mg/ml, the *E. floccosum* cells were all killed in 3 hours only. The 1 μg/ml of the control drug inhibited the fungal cells in 48 hours. When *Coleus* spp. and *E. abyssinica* extracts were combined and the activity compared to *Coleus* spp. extract alone, it was synergistic against *E. floccosum* in the time kill assay, the combinations also showed synergy on *E. floccosum* only, it showed additive or antagonistic activity on the rest of the tested fungi. In the checker board assay, *E. abyssinica* and *Coleus* spp. extracts showed synergistic effect against *T. mentagrophytes*. Another synergistic effects were also observed with *M. gypseum* at four different combinations of *E. abyssinica* and *Coleus* spp extracts proportions, but *C. albicans* showed some significant level of antagonism to the various tested combinations” [29].

“The leaves of *Dissotis multiflora* (Melastomataceae) and leaves of *Paullinia pinnata* (Sapindaceae) were macerated in ethanol 95 % and assayed with Agar- well diffusion method against six fungal species, including *Candida krusei*, *C. tropicalis*, *C. parapsilosis*, *C. haemulunii*, *C. lipoelytica* and *C. albicans* alongside with Fluconazole and nystatin standards. Inhibition zone diameters, MIC and MFC were calculated. The MFC/ MIC ratio showed that the methanolic fractions of *D. multiflora* and *P. pinnata* have a fungicidal action on all 6 species. Generally, the inhibition zone diameters were ranging from 10.33 mm to 19 mm, while the MICs and MFCs of the actives extracts were ranged respectively from 0.78 to 12.5 mg/ml and 1.56 to 25 mg/ml. The combinations showed significant antifungal activity compared to those of the individual fractions, as the combination of methanolic fractions of *D. multiflora* and *P. pinnata* showed a synergistic effect against *C. krusei* and *C. albicans* [24].

*Allium sativum* (Alliaceae) and *Nigella sativa* (Ranunculaceae) were tested together and each one alone and compared with fluconazole alone against *Candida albicans*, the first plant was extracted with distilled water while the second plant was extracted by ethanol. The results indicated that *N. sativa* alone has no antifungal activity (up to 10% concentration), and when combined with *A. sativum* it has weak antifungal activity against *C. albicans* when compared to *A. sativum* alone. Again, *A. sativum* extract with *N. sativa* caused an increase in the zone of inhibition against *C. albicans* when compared to fluconazole alone. The study concluded that *A. sativum* extract has significant effect on *C. albicans*, *N. sativa* doesn’t has anti-candidal activity on *C. albicans*, the synergistic effect of *N. sativa* extract with *A. sativum* extract has less anti-candidal activity than *A. sativum* extract alone but more activity when compared with fluconazole alone [30].

The antifungal activity of about 50 plants were tested individually and in combinations against *Fusarium oxysporum* f. sp. *Ciceros*, a causal organism of *Fusarium* wilt of chickpea. Differential activity was observed against mycelium growth. Generally, the combined roots decoction extract of *Acacia catechu* (Mimosaceae) and leaf decoction extracts of *Lowsonia alba* (Lythraceae)(combined in ratio 1:1) showed greatest activity than their individual use (86.42%), while the percentages of mycelium growth inhibition of both *A. catechu* and *L. alba* extracts were 73.58% and 82.54% respectively [31].

### 3.2 Plant(s) – Drug(s) Combinations

The following table (Table 2) shows some studies which had tested a combination of a plant extract(s) with Known antifungal agents, and showed if there is any synergism, indifferent, additive or antagonism effects.

The *Silybum marianum* (Asteraceae) seeds were extracted with water. Then the effect of the extract was investigated both individually and in combination with fluconazole, against drug-resistant clinical isolates of *Candida albicans* and *C. glabrata*. The mean MIC₉₀ of fluconazole against *C. albicans* and *C. glabrata* were determined at 512 μg/ml and the MIC₉₀ of *S. marianum* was 2,048 μg/ml. After combination, the MIC₉₀ of *S. marianum* and fluconazole was 128 μg/ml. Therefore, the aqueous extract of *S. marianum* in combination with fluconazole was more potent in vitro when compared with each one alone [32].

The leaves, twigs and stem of *Uvaria angolensis*, *U. muricata* (Annonaceae) and *Terminalia catappa* (Combretaceae) were extracted by water and ethanol. The extracts were evaluated each alone and when combined with nystatin and ketoconazole against yeasts species isolated from HIV patients by using agar dilution method. Broth micro dilution method and subculture were used to determine their antifungal parameters (MIC and MFC). The results showed that the
Table 2. Some examples plant extract(s) combined with known antifungal agents; and the type of interactions which they cause

<table>
<thead>
<tr>
<th>Latin name of plant extracts used</th>
<th>Antifungal agent used</th>
<th>Fungal organism(s) used</th>
<th>Type of interaction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Terminalia catappa</em> aerial parts of hydro-alcoholic extract</td>
<td>Nystatin and ketoconazole (each alone)</td>
<td><em>Candida albicans</em></td>
<td>Synergism</td>
<td>[33]</td>
</tr>
<tr>
<td><em>Allium sativum</em> ethyl acetate extract</td>
<td>Amphotericin B</td>
<td><em>Cryptococcus neoformans</em></td>
<td>Synergism</td>
<td>[34]</td>
</tr>
<tr>
<td><em>Astronium urundeuva</em> hydro-ethanolic extract</td>
<td>Amphotericin B</td>
<td><em>Candida albicans</em></td>
<td>Synergism</td>
<td>[35]</td>
</tr>
<tr>
<td><em>Uncaria tomentosa</em> stem barks water insoluble fraction of hydro-ethanolic extract</td>
<td>Terbinafine</td>
<td><em>Candida krusei</em> and <em>Candida glabrata</em> clinical resistant strains</td>
<td>Synergism</td>
<td>[38]</td>
</tr>
<tr>
<td><em>Uncaria tomentosa</em> stem barks water insoluble fraction of hydro-ethanolic extract</td>
<td>Fluconazole</td>
<td><em>Candida krusei</em> and <em>Candida glabrata</em> clinical resistant strains</td>
<td>Additive</td>
<td>[38]</td>
</tr>
<tr>
<td><em>Ocimum basilicum</em> leaves ethanol extract</td>
<td>Amphotericin B</td>
<td><em>Cryptococcus neoformans</em> and <em>C. gattii</em></td>
<td>Synergism</td>
<td>[39]</td>
</tr>
<tr>
<td><em>Ocimum basilicum</em> leaves hexane fraction of ethanol extract</td>
<td>Amphotericin B</td>
<td><em>Cryptococcus neoformans</em> and <em>C. gattii</em></td>
<td>Additive</td>
<td>[39]</td>
</tr>
<tr>
<td><em>Rubus chingii</em> fruits ethanolic extract</td>
<td>Fluconazole</td>
<td>Fluoconazole-resistant <em>Candida albicans</em></td>
<td>Synergism</td>
<td>[41]</td>
</tr>
<tr>
<td><em>Acmella caulirhiza</em> hexane extract</td>
<td>Amphotericin B</td>
<td><em>Candida krusei</em> and <em>C. orientarlis</em></td>
<td>Synergism</td>
<td>[48]</td>
</tr>
<tr>
<td><em>Acmella caulirhiza</em> hexane extract</td>
<td>Amphotericin B</td>
<td><em>Candida albicans</em>, <em>C. duabushaemulonii</em>, <em>C. haemulonii</em>, <em>C. auris</em> and <em>C. famata</em></td>
<td>Synergism</td>
<td>[48]</td>
</tr>
<tr>
<td><em>Acmella caulirhiza</em> hexane extract</td>
<td>Clotrimazole</td>
<td><em>Candida albicans</em>, <em>C. krusei</em> and <em>C. orientarlis</em></td>
<td>Synergism</td>
<td>[48]</td>
</tr>
<tr>
<td><em>Acmella caulirhiza</em> hexane extract</td>
<td>Clotrimazole</td>
<td><em>Candida duabushaemulonii</em>, <em>C. haemulonii</em>, <em>C. auris</em> and <em>C. famata</em></td>
<td>Antagonism</td>
<td>[48]</td>
</tr>
</tbody>
</table>
leaves extract of *T. catappa* showed the best antifungal activity (MIC = 1.56 mg/ ml, 0.78 mg/ ml and 0.78 mg/ ml on *Candida albicans*, *Cryptococcus neoformans* and *Candida parapsilosis* respectively). The most active extract was combined with nystatin and ketoconazole, presented synergistic effects with the best index being FIC Index of 0.17 ± 0.09 from *T. catappa* extract on *C. albicans* and a significant reduction of the MIC values of the extracts, nystatin (3 to 1,600 times) and ketoconazole (2 to 512 times); these synergistic results support the traditional use of these plants and suggest that they could serve as potential sources of antifungal agents [33].

An extract of garlic (*Allium sativum*, Liliaceae) was prepared with ethyl acetate. Minimal inhibitory concentrations (MIC) of *A. sativum* extract and amphotericin B were determined by a broth dilution technique method, against three clinical isolates of *Cryptococcus neoformans*. It was found that *A. sativum* possessed potent in vitro fungistatic and fungicidal activities against the three isolates of *C. neoformans*, the MIC of the garlic extract was ranging between 6.1–12.2 μg/ml, while the MIC of amphotericin B against the three isolates was ranging between 0.1–0.2 μg/ml. The MFC was 12.2 μg/ml and 0.2–0.4 μg/ml for *A. sativum* extract and amphotericin B respectively. In combination study, *A. sativum* extract and amphotericin B showed synergistic effect, as the fractional inhibitory concentration indices were 0.5. The level of synergy was comparable to that combination of amphotericin B and flucytosine [34].

*Astronium urundeava* (Anacardiaceae) leaves were exhaustively percolated by hydro-ethanol. *In vitro* susceptibility test to examine the anti-fungal activity was carried out against clinical isolates strains of *Candida albicans* and *C. glabrata*. Minimum fungicidal concentration (MIC) was also calculated, and finally determination of the activity of the free extract in combination with clinically used anti-fungal agents. Individual assays for each drug alone demonstrated the anti-fungal activity of the free extract against both *Candida* species, with increased activity against *C. glabrata*, including collected strains and clinical isolates displaying different levels of resistance against the most common clinically used anti-fungal drugs. In the checkerboard assays for combination study, different concentrations of the plant extract were used in combination with different dilutions of fluconazole, caspofungin and amphotericin B, a majority of combinations showed indifference effect, with the notable exemption of the combinations between the plant extract and amphotericin B against *C. albicans* which resulted in synergism, this will lead to an increase of activity and a reduce of the side effects of amphotericin B, which is mainly nephrotoxicity [35].

A flower of *Flos Rosa Chinensis* (Rosaceae) was extracted with ethanol 70%, the extract was used to examine the anti-fungal activity combined with fluconazole against thirteen clinical isolates of *Candida albicans* resistant strains to fluconazole. The minimum inhibitory concentration (MIC) of the extract was determined using a checkerboard micro dilution assay. *R. chinensis* alone exerted efficient anti-fungal activity with MIC ranging from 20 μg/ml to 40 μg/ml, the plant extract failed to enhance the effects of fluconazole against sensitive *C. albicans* strains, although it rendered fluconazole-resistant *C. albicans* more sensitive! By *in vivo* studies, the *R. chinensis* anti-fungal mechanism showed that it strengthens fluconazole to inhibit the action of ergosterol biosynthesis by promoting the transformation of lanosterol to eburicol, suggesting that the anti-fungal mechanism of action involves the inhibition of ergosterol biosynthesis [36].

*In vitro* anti-fungal activity of three fractions obtained from the aqueous extract of *Acca sellowiana* (Myrtaceae) leaves was tested against resistant strains of non-albicans *Candida*. Its reversal of fluconazole resistance was also tested by combining it with the plant fractions. The anti-fungal activity of the three fractions (F1, F2 and F3) was tested at 500 μg/ml by micro dilution method. *C. glabrata* showed the lowest MIC values (500–3.90 μg/ml), and among all fractions F2 was the most effective. Checkerboard assay was applied to determine the effect of the combination of the F2 fraction with fluconazole, the combination showed FICI of ≤0.5 (synergism) against the *C. glabrata* resistant isolates. This study suggests that the combination of F2 and fluconazole might be used as an alternative treatment for mucocutaneous infections caused by resistant strains of non-albicans *Candida* species [37].

*Uncaria tomentosa* (Rubiaceae) stem barks were extracted with ethanol 50%, and the extract was tested for *in vitro* synergism test of its water insoluble fraction in combination with fluconazole...
and terbinafine (FLZ and TRB), the organisms used were resistant non *Candida albicans* isolates. Both checkerboard method and microdilution technique were used in this study. The isolates were *Candida krusei* ATCC 6258, CK01, CK04 and *Candida glabrata* CG40039, CG10, RL02, RL03. The results indicated that TRB and FLZ when tested alone up to a 64 μg/ ml were unable to inhibit the growth of all isolated strains. But the addition of water insoluble fraction to TRB resulted in enhancement of the anti-fungal activity of TRB, the combined concentration ratio was 8:1.95 μg/ ml concentration ratio, this enhancement activity was revealed on CK6258 terbinafine resistant isolate (88%), while the same isolate with TRB and water insoluble fraction alone showed only 40.7% and 20% respectively; TRB and water insoluble fraction of the plant extract at concentration ratio 4:1.95 μg/ ml caused significant cell damage (79.52%) regarding the CK04 isolate, the same combination was able to induce a significant synergic effect on nearly all isolates. On the other hand, regarding FLZ resistant isolate of CK04, a cell damage of about 80% was noticed for TRB and water insoluble fraction combined in a concentration ratio of 1.95:8 μg/ ml, in that case the individuals alone showed only 50% of cell damage below using the same concentration ratio. To conclude, the combinations of the water insoluble fraction of the plant extract with either TRB or FLZ were able to reduce the MIC values (either additive or synergic effects were clearly noticed in all tested isolates); while for the plant fraction combination with TRB showed synergistic effect in four different isolates (two *C. krusei* and two *C. glabrata*); but also for the combination of the plant fraction and FLZ was active only in three isolates (one *C. krusei* and two *C. glabrata*); in other isolates an additive effects were observed [38].

Absolute ethanol was used to prepare an extract from leaves of *Ocimum basilicum* (Lamiaceae), then the extract was suspended in distilled water and fractioned successively beginning with n-hexane, dichloromethane, ethyl acetate and ending with n-butanol. Also an essential oil was obtained from the plant. All these plant products were examined for anti-cryptococcal activity against three clinical strains of *Cryptococcus neoformans* T444, C. neoformans H99 and *C. gattii* WM779; also combinations were prepared using amphotericin B in which the FIC index values were ranging between 0.187 to 0.75, this showed that all these combinations reduced the MIC values. The synergistic effect was observed in the combination of amphotericin B and the ethanol crude extract (reducing their MIC from 1.56 to 0.099 μg/ml and 625 to 78 μg/ml respectively); in the combination of ethanol crude extract with essential oil, it was observed that there was a reduction in their MIC values from 625 to 39 μg/ml and 1,250 to 157.2 μg/ml respectively, and in the combination of hexane fraction and essential oil, it was observed a reduction in their MIC values from 156 to 20 μg/ml and 1,250 to 78.72 μg/ml respectively. When amphotericin B was combined with 78 μg/ml with hexane fraction, their MIC values were reduced from 1.56 to 0.396. Most of combinations were synergistic, only the combination of amphotericin B with hexane fraction was additive [39].

Leaves of *Eugenia uniflora* (Myrtaceae) were extracted by maceration with 95% ethanol. The extract was then diluted by DMSO, then it was assayed for anti-fungal activity, either alone or combined with amphotericin B, rimexolone, nystatin and metronidazole against *Candida albicans*, *C. krusei* and *C. tropicalis*. The MIC was >1,024 μg/ml. However, an interesting potentiation of the anti-fungal activity was demonstrated when *E. uniflora* alcoholic extract was combined with metronidazole against *C. tropicalis*, as it lowered the MIC four times (from 128 to 32), no synergistic activity against the other species was seen. The study concluded that *E. uniflora* appears to be promising in the development of therapies, mainly due to its low toxicity in vitro, which allows to proceed with in vivo studies for drug evaluation [40].

*Rubus chingii* (Rosaceae) fruit powder was extracted with 70% ethanol and investigated for the anti-fungal activity in combination with flucytosine against *Candida albicans*. The growth curves for *C. albicans* after treatment with *R. chingii* extract, flucytosine alone and a combination were all constructed. Both *R. chingii* extract and flucytosine alone didn’t show significant anti-fungal activity, but the two drugs when combined together showed significant synergy; the MIC80 for flucytosine was >256 mg/ml and for *R. chingii* extract was >5,000 mg/ml; the MIC80 for both combined drugs was only 0.0625–16 mg/ml for flucytosine and 4.88–312.5 mg/ml for *R. chingii* extract [41].

The aerial parts of *Sarcococca saligna* (Buxaceae) plant was percolated with absolute ethanol. The antifungal activity was determined.
by disk diffusion method, for *S. saligna* extract and its combination effect with fluconazole, the tested organisms were *Aspergillus Species* (*A. niger, A. treus, A. flavus* and *A. Fumigates*) on Sabouraud dextrose agar. The activity was measured in form of zone of inhibition. No clear zones of inhibition were observed for all test strains around standard fluconazole paper disks, and this indicates that these test strains were resistant to fluconazole. The *S. saligna* extract showed anti-fungal activity (MIC ≥ 0.5 mg/ disk) against *A. niger and A. treus*, the anti-fungal activity was dose-dependent. But *S. saligna* extract did not show any activity against *A. flavus* at contents used for the bioassay (0.5, 1, 2, 3 and 4 mg/ disk), also another tested strain (*A. fumigates*) was less susceptible to *S. saligna* extract compared with *A. niger and A. treus*. The combination effect of this plant extract at the same amounts (0.5, 1, 2, 3 and 4 mg/ disk) with fluconazole (25 μg/ disk) was investigated, it was reported that the ethanol extract of *S. saligna* enhanced the anti-fungal activity of fluconazole against *A. niger, A. treus and A. flavus*, at the highest tested contents of 4 mg/ disk, 1.15-, 0.64- and 2.47- fold increases in inhibition zone surface area were observed for *A. niger, A. treus* and *A. flavus* respectively which indicate synergism. However, no enhancing effect was observed by the plant extract against *A. fumigates* at tested concentrations of the extract [42].

*Hippophae rhamnoides* (Elaeagnaceae) twigs and leaves were each extracted with methanol 80%. The extracts were then studied for their anti-candidal activity of each extract alone and in combination with either fluconazole or caspofungin. The MIC were determined using two different methods (micro dilution broth assay and agar dilution assay). In both methods, *H. rhamnoides* extracts were dissolved in DMSO 50% and their MIC values against *C. albicans* were established as 250 mg/ ml and 31.5 mg/ ml for twigs and leaves extracts respectively, unexpectedly, the growth of the blood isolated strain of *C. glabrata* was inhibited by the twigs extract at a relatively low concentration (15.6 mg/ ml) and by the leaves extract at a concentration as low as 3.9 mg/ ml. In the combination study, the findings indicate that the MIC values of fluconazole and caspofungin were decreased, so these extracts preparations increased fluconazole activity against both *C. albicans* and *C. glabrata*, thus the plant extracts have a good potential for the development of novel antifungal products supporting classic drugs [43].

The dried fruits of *Terminalia chebula* (Combretaceae) were extracted with methanol. *In vitro* anti-fungal activity was studied against *Candida albicans* by the agar well diffusion method. Also the combination of this plant extract with amphotericin B was studied as equal volumes (25 μl) of each was added in the well and the zone of inhibition was measured. The results revealed that the plant extract had not showed any inhibition at concentration of 10 mg/ ml and 30 mg/ ml, but at the same concentrations, amphotericin B was susceptible against *C. albicans*. When the plant extract was combined with amphotericin B, the zone of inhibition had been increased significantly. The study concluded that the enhancement of anti-fungal activity of amphotericin B could be explained by the presence of biologically active compounds which are present in the plant extract. Thus, the combination of *T. chebula* extract with amphotericin B could be beneficial to increase the anti-fungal activity against *C. albicans* [44].

Seeds powder of *Pimpinella anisum* (Apiaceae) and *Moringa oleifera* (Moringaceae) leaves were extracted separately with distilled water. 250 μg/ml of terbinafine (anti-fungal agent) was dissolved in DMSO. MIC of the individual drug and its combination with plant extracts were calculated against the pathogenic *Microsporum canis*. Terbinafine has a MIC of 6 μg/ml, whereas *M. oleifera* and *P. anisum* extracts have a MIC of 80 mg/ ml and 60 mg/ ml, respectively. A combination of terbinafine, *M. oleifera* and *P. anisum* had the greatest effect in inhibiting the development of the pathogenic fungi. In comparison to the control experiment, all combinations were found to have a considerable impact on the growth of *M. canis* throughout the experiment. In addition, all the treatments comprising terbinafine and a plant extract has a higher inhibitory effect compared to combination of plant extracts (*M. oleifera* in combination with *P. anisum*) treatments and the control experiment. This implies that the antifungal activity of terbinafine is enhanced when used in a combination treatment with *M. oleifera* and *P. anisum* extracts [45].

The leaves of *Vernonia adenosis* (Asteraceae) was successively extracted by hexane, dichloromethane, ethyl acetate, dichloromethane/ methanol, ethanol, methanol and water. All extracts were tested against inhibiting the growth of *Candida krusei*. The effects of combining fluconazole and the most potent extract were
also examined. The MIC of fluconazole against *C. albicans* was found to be 8 μg/ ml, while its MIC against *C. krusei* was 125 μg/ ml. The MFC for fluconazole on *C. krusei* was also 125 μg/ ml, so, *C. krusei* was somehow resistant and less sensitive to fluconazole when compared with *C. albicans*. Therefore, subsequent work was then conducted on *C. krusei* only. The results for the effect of all tested *V. adoensis* extracts imply that all extracts (except the water extract) had no effect on inhibiting the growth of *C. krusei*, as the cell densities were still high. Hence distilled water extract of *V. adoensis* significantly reduced the fungi growth, so it was combined with fluconazole to determine if there was any enhanced effects, the concentrations of fluconazole were ranged between 500 μg/ ml to 8μg/ ml, and the concentrations of water extract were ranged between 100 μg/ ml to 12.5 μg/ ml, it was observed that the MIC of the combination of 100 μg/ ml of the water extract with 32 μg/ ml of fluconazole was achieved, as the combination lowered the MIC of fluconazole on *C. krusei* from 125 μg/ ml to 32 μg/ ml, also it was observed that the cell densities of the fungi decreased as the concentration of water extract increased, and decreased with a greater decrease observed at higher concentrations of fluconazole. The study concluded that combining different concentrations of fluconazole with 100 μg/ ml of the water extract increased the potency of fluconazole [46].

A randomized controlled clinical trial was applied to determine the effect of *Salvia officinalis* (Lamiaeae) extract as vaginal tablets alone, and its effect in combination with clotrimazole on the recovery of vulvo-vaginal candidiasis and finally to compare its effectiveness. 111 participants were randomly divided into three groups of 37 patients using block randomization with block sizes of 6 and 9, and allocation ratio of 1:1:1:1, group one was treated with 100 mg vaginal tablet of clotrimazole and placebo (CP), group two was treated with 400 mg vaginal tablet of *S. officinalis* extract and placebo (SP), and group three was treated with vaginal tablet of *S. officinalis* extract and clotrimazole (SC), all once daily for 7 days; on the seventh day, vulvo-vaginal candidiasis was examined by vaginal symptoms and wet test, and if positive, it was examined by culture in chrome agar *Candida* medium. The frequency of a positive wet test confirmed by sabrodextrose agar medium 7 days after treatment was significantly lower in the third group taking *S. officinalis* and clotrimazole than the reference second group of *S. officinalis* and placebo. There was no significant difference in the group taking placebo with either *S. officinalis* or clotrimazole. This made conclusion that *S. officinalis* in the form of vaginal tablet, alone and when combined with clotrimazole, can treat the vulvo-vaginal candidiasis [47].

*Acmella caulirhiza* (Asteraceae) and *Senna didymobotrya* (Fabaceae) extracts were tested against *Candida* spp., hexane and methanol extracts were prepared by maceration from each extract. Clotrimazole, ketoconazole, nystatin, amphotericin B and griseofulvin were dissolved in DMSO. Methanol and hexane extracts of *A. caulirhiza* and *S. didymobotrya* were also weighed and dissolved in DMSO to give stock solutions. The test organisms (*Candida* spp.) that were used are: *Candida albicans*, *C. duabushaemulonii*, *C. haemulonii*, *C. auris*, *C. famata*, *C. orientaris* and *C. krusei*. MIC values were determined by broth micro-dilution test and was found that griseofulvin, clotrimazole and ketoconazole produced high MIC values in comparison to the control drug; for nystatin, however, the results were erratic with growth at low concentration and no zone of inhibition at higher concentrations meaning that all the pathogens died. From the plant extract/ conventional drug concentration gradients most of the combinations showed MIC values at lower conventional drug concentration and higher extract concentration; two combinations, however, amphotericin B/ *A. caulirhiza* methanol extract and ketoconazole/ *S. didymobotrya* hexane extract deviated from this observation where the extract concentration was lower than the conventional drug concentration; amphotericin B/ *A. caulirhiza* hexane extract combination was synergistic when used against *C. krusei* and *C. orientaris*; while with the other *Candida* species it was antagonistic. Clotrimazole/ *A. caulirhiza* hexane extract combination was synergistic against *C. albicans*, *C. krusei* and *C. orientaris* but antagonistic against the other *Candida* species. The other combinations were indifferent and antagonistic against the *Candida* species used. This study found that *A. caulirhiza* and *S. didymobotrya* have potent anti-fungal phytochemicals and thus *A. caulirhiza* extract modulates clotrimazole. It is thus recommended that pure active anti-fungal components of these plants be determined and pure active components of *A. caulirhiza* be used to develop new anti-fungal regimens in combination with clotrimazole [48].
The dried aerial parts of *Echinophora platyloba* (Apiaceae) were macerated by ethanol 70%. Three different concentrations of the ethanolic extract were prepared (4, 5.2, and 11%). The antimicrobial and anti-fungal activities of each concentration alone was evaluated against *Candida albicans* by agar dilution and micro broth dilution assays. The susceptibility of *C. albicans* (MIC and MLC) and the corresponding size of zone of inhibition to different types and concentrations of *E. platyloba* and amphotericin B each one alone and in combination by disc diffusion method were also observed. *C. albicans* growth was inhibited by concentrations ≥ 2 mg/ml of the plant extract (2, 4, 8, 16, 32, 64, 128 and 256 mg/ml), also there was a 50% reduction in MIC and a 75% reduction in MLC values of the mixture of amphotericin B and 5% ethanolic extract against *C. albicans* in comparison to amphotericin B alone; the zone of inhibition of the mixture showed 22% increase in diameter in comparison to that of amphotericin B alone. Therefore, the most potent anti-fungal agent was the mixture of ethanolic extract 5% plus amphotericin B, followed by amphotericin B alone, ethanolic extract 5% alone, ethanolic extract 11% alone, ethanolic extract 4% and lastly ethanol 70% in descending order. It was clear that *E. platyloba* showed potent anti-fungal activity, its inhibitory action against *C. albicans* was the highest and some degrees of synergy was recorded in combination of amphotericin B plus *E. platyloba* 5% ethanolic extract, the study suggested that the synergistic effect of this mixture needs further in vivo studies to evaluate its actual effect [49].

The effects of the aqueous and methanol extracts of green tea leaves (*Camellia sinensis*, Theaceae) and the synergistic effects of these two extracts were studied along with two drugs (i.e. itraconazole and voriconazole) against four strains of *Aspergillus* species. Micro dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The results concluded that two out of four strains of (i.e. *A. flavus* and *A. terreus*) were sensitive to itraconazole, while the other two strains were resistant; the results also showed that all four strains were resistant to voriconazole. The aqueous and methanol extracts of green tea did not showed anti-fungal activity when tested individually, but the aqueous extract of green tea when combined with itraconazole were synergistic against *A. niger* and *A. fumigatus*. Also, the combined methanol extracts of green tea and itraconazole against *A. niger* and the combined aqueous extracts of green tea and voriconazole against *A. flavus* and *A. fumigatus* were reported of valuable effect. The study concluded that there is a valuable synergistic effects between the tested anti-fungal drugs and both extracts [50].

In the study to find the effect of *Hibiscus sabdariffa* (Malvaceae) roselle extract in synergism with voriconazole and fluconazole against fluconazole-resistant *Candida albicans* isolates, air-dried calyces were extracted using 80% hydro-methanol. Determination was done by plate antimicrobial susceptibility testing method. Checkerboard assays were applied to find the interaction of *H. sabdariffa* extract with fluconazole and voriconazole. When the extract was tested alone, the MIC values were ranged from 0.5 to 2 mg/ml. All the isolated strains showed resistance to fluconazole (MIC > 16 µg/ml). All the isolated strains showed susceptibility to voriconazole with MICs values of < 0.016 µg/ml. The results from the checkerboard assay indicate that the combinations of fluconazole with the extract have an ‘indifference’ effect; generally, the results revealed that combinations of fluconazole and *H. sabdariffa* have no synergism except for one strain among the six tested strains. In the case of voriconazole, when the extract was combined with voriconazole at different concentrations, there was a reduction of the MIC values of voriconazole, in almost all of the six strains, there was a strong synergistic effect demonstrated by FICI calculations [51].

The aerial parts of the six selected plants [i.e. *Atriplex halimus* (Amaranthaceae), *Alhagi maurorum* (Fabaceae), *Brassica tournefortii* (Brassicaceae), *Nicotiana glauca* (Solanaceae), *Mesembryanthemum crystallinum* (Aizoaceae) and *Peganum harmala* (Zygophyllaceae)] were extracted with aqueous and other organic solvents (i.e. petroleum ether, chloroform, ethyl acetate and methanol). Then they were screened against the different human pathogenic fungal species (i.e. *Candida albicans*, *C. tropicalis*, *Trichosporon spp*, *Aspergillus fumigatus*, *A. flavus* and *A. versicolor*) either alone or in combination between them. The result revealed that petroleum ether fractions of *M. crystallinum*, *N. glauca*, *P. harmala*, *A. halimus* and *B. tournefortii* were the most active fractions compared with chloroform, ethyl acetate and methanol fractions, and also with their crude ethanolic extracts. The MIC values of active
fractions were ranged between 0.195 and 6.25mg/ml, whereas the fungicidal activity was ranged between 0.781–12.5 mg/ml. The most efficient anti-fungal activity was showed by the petroleum ether fraction of *M. crystallinum* which inhibited the growth of yeast at MIC value of 0.195 mg/ml and moulds at MIC values ranged from 1.56–3.12 mg/ml. The synergistic effect of the most active fractions with fluconazole were tested by using well diffusion assay, the majority of combinations between one plant extract with another plant extract showed synergistic effect (for example petroleum ether fraction of *B. tournefortii* with *A. halimus*); also the majority of combinations between plant extracts and fluconazole showed even highly synergistic effect against all tested fungal species except the combination between *A. maurorum* extract and fluconazole which showed strong antagonistic effect against *A. fumigatus* and *A. versicolor*. There was a strong synergistic effect against *C. albicans* and *C. tropicalis*, a slight synergistic effect against *Tricosporon* spp and *A. versicolor*, and lastly antagonistic effect against *A. flavus* [52].

The leaves of *Chromolaena odorata* (Asteraceae) were macerated in chloroform. The extract was then individually evaluated for its anti-fungal activity against *Aspergillus niger* using agar cup diffusion technique; while checkerboard technique was applied for evaluation its after combination with itraconazole. The results displayed that *A. niger* was sensitive to itraconazole (MIC = 0.0002 mg/ml) and also sensitive to the chloroform extract of *C. odorata* (MIC = 5 mg/ml). Their combination revealed that some concentration ratio showed additive properties, while some other ratios were synergistic, also indifferent properties were seen in some concentration ratios; the synergistic effect of *C. odorata* leaf chloroform extract and itraconazole was recorded in 4 ratios (i.e. 9:1, 8:2, 7:3 and 5:5), the ratio 5:5 produced the most synergistic effect. In the drug ratio that showed the greatest synergy (5:5), the MIC values of itraconazole and *C. odorata* extract were reduced by 20 and 8 times respectively, the interpretation is that *C. odorata* chloroform extract modified itraconazole activity to a much larger extent than does itraconazole alone [53].

The aerial parts of *Tanacetum vulgare* (Asteraceae) was extracted by ethyl acetate at room temperature. The anti-fungal activity of the ethyl acetate extract, chlorhexidine and sodium hypochlorite were tasted each one alone and in combination between them against *Candida albicans*. The results was displayed as size of zone of inhibition. The inhibition zone of chlorhexidine was 30.3–19.3 mm, but in combination with ethyl acetate extract of *T. vulgare* (100 mg/ml) the inhibition was from 32.7–30 mm, indicating that this combination exerted a marked synergistic effect against *C. albicans*. In addition, the inhibition zone of sodium hypochlorite was between 69.7–65 mm, which was higher than the inhibition zones of ethyl acetate extract and chlorhexidine, the combination of ethyl acetate extract with sodium hypochlorite resulted in a loss of anti-fungal activity [54].

The combination of antifungal creams with some selected natural products from plants was applied against some fungal dermal infections which were resistant with some antifungal agents, the antifungal creams used were clotrimazole, fluconazole, ketoconazole and terbinafine, they were combined with either turmeric rhizomes essential oil (*Curcuma longa*, Zingiberaceae) or *Aloe vera* (Asphodelaceae) gel; the tested species were *Candida albicans*, *Penicillium notatum*, *Aspergillus* fumigatus, *A. niger*, *A. flavus*, *Trichophyton rubrum*, *T. violceum* and *T. mentagrophytes*. The antifungal activity was carried out using agar well diffusion method. GC-MS was applied to know the phytochemical constituents in both extracts, it showed 36 and 18 bioactive compounds in *C. longa* essential oil and *Aloe vera* gel respectively, these phytochemical compounds were related to phenols, flavonoids, saponins, alkaloids, steroids, terpenoids and cardiac glucosides. All antifungal creams applied in this study revealed zones of inhibition with values ranged from 5 to 14.3 mm, the *C. longa* essential oil alone was 5 to 11 mm, while *Aloe vera* gel alone was ranged from 8 to 11.7 mm; the MIC values of antifungal creams, *C. longa* essential oil and *Aloe vera* gel were between 1.25 to 10 mg/ml; the combination of antifungal creams with either *C. longa* essential oil or *Aloe vera* gel revealed synergistic and indifferent properties (i.e. clotrimazole + *C. longa* E.O. against *C. albicans*, ketoconazole + *C. longa* E.O. against *A. niger*, terbinafine + *C. longa* E.O. against *C. albicans*, clotrimazole + *Aloe vera* gel against *C. albicans*, fluconazole + *C. longa* E.O. against *A. flavus* and terbinafine + *Aloe vera* gel against *C. tropicalis*), all displayed synergistic properties, while other combinations were indifferent without antagonism [55].
3.3 Essential Oil(s) – Drug(s) Combinations

The following table (Table 3) shows some studies which had tested a combination of a plant’s essential oil with known antifungal agents, and showed if there is any synergism, indifferent, additive or antagonism effects.

Essential oils obtained from Cinnamomum cassia (Lauraceae), Melaleuca alternifolia (Myrtaceae), Mentha piperita (Lamiaceae), Origanum vulgare (Lamiaceae) and Syzygium aromaticum (Myrtaceae) were tested against 19 strains of Malassezia pachydermatis isolated from healthy dogs. The anti-fungal activity was determined by checkerboard assay to search for interactions between these essential oils and some known anti-fungal drugs; the combination concentrations of clotrimazole used was ranged from 0.0625 μg/ ml to 32 μg/ ml, while the essential oils concentrations used were as follow: C. cassia (0.156- 20 mg/ ml), S. aromaticum (0.156- 20 mg/ ml), M. piperita (0.4- 50 mg/ ml), O. vulgare (0.4 - 50 mg/ ml) and M. alternifolia (0.4- 50 mg/ ml). The fractional inhibitory concentration indices (FICI) of clotrimazole combined with selected essential oils were calculated. The combinations of clotrimazole with either M. alternifolia, M. piperita or O. vulgare essential oils displayed synergistic effect. The combinations of clotrimazole with either C. cassia or S. aromaticum essential oils showed indifferent effect. The combinations of clotrimazole with either S. aromaticum or M. alternifolia essential oils revealed an additive effect [56].

An essential oil from Myrtus communis (Myrtaceae) was evaluated for anti-fungal activity individually and in combination with amphotericin B against Candida albicans and different species of Aspergillus spp(i.e. A. niger, A. parasiticus & six isolates of A. flavus) using broth micro dilution assay. MIC and MLC values of amphotericin B were 1- 2 and 2 mg/ ml respectively for C. albicans, and their values were 4- 8 and 8 mg/ ml respectively for Aspergillus spp. Even the MICs of the tested plant’s essential oil did not differ so much, as the MIC and MLC values were ranged between 8- 16 and 16- 32 ml/ ml respectively against the tested fungi. Checkboard micro titer test was used to examine the antifungal activity of amphotericin B with the essential oil, it was observed that the MIC value of amphotericin B against C. albicans was lowered from 2 to 0.06 mg/ ml after the addition of essential oil at dose of 4 ml/ml; the FIC values of the essential oil combined with amphotericin B were 0.25 ml/ml and 0.03 mg/ ml for amphotericin B alone against C. albicans, while against A. niger were 0.25 ml/ml and 0.015 mg/ ml respectively, the FIC index showed marked synergism against both C. albicans and A. niger [61].

The essential oils of the following plants were tested [Mentha piperita (Lamiaceae), Carum coticum (Apiaceae), Cinnamomum verum (Lauraceae), Syzygium aromaticum (Myrtaceae), Cymbopogon martini (Poaceae) and Thymus vulgaris (Lamiaceae)] and their active compounds were also tested (such as thymol, cinnamaldehyde, eugenol and geraniol), also their in vitro interaction with fluconazole against drug-resistant pathogenic fungi including (Aspergillus niger, A. fumigatus, A. solani and Trichophyton rubrum) was tested. All examined essential oils were active (MIC range: 72– 288 μg/ ml; MFC range: 144– 576 μg/ ml). The essential oil of M. piperita was strongly active against A. fumigatus and moderately active against T. rubrum (MIC value = 288 and 576 μg/ ml respectively). Cinnamaldehyde, eugenol and geraniol showed antifungal activity higher than essential oils (MIC values ranged from 40- 160 μg/ ml, and MFC values ranged from 80- 320 μg/ ml); cinnamaldehyde was the most active against A. solani and T. rubrum. In combination study, all the tested essential oils and active compounds showed significant levels of synergistic interaction with fluconazole against T. rubrum, the essential oils of S. aromaticum (0.250), eugenol (0.375) and cinnamaldehyde (0.187) exhibited synergistic interactions with fluconazole against A. fumigatus but no interactions were observed for the oils of C. martini and geraniol with fluconazole, Cinnamaldehyde was the most effective in combination therapy as it reduced the MIC of fluconazole up to 8 fold against both A. fumigatus and T. rubrum. The highest reduction in MIC (i.e. 128 fold) was recorded for oil of S. aromaticum in combination with fluconazole against T. rubrum. No combination was found to be antagonistic [62].

A study was conducted to test fluconazole against 32 clinical strains of fluconazole-resistant Candida albicans after their exposure to sub-lethal concentrations of tea tree oil (TTO) distilled from Melaleuca alternifolia (Myrtaceae) leaves or its main active constituent terpinen-4-ol using broth macro dilution. C. albicans strains tested to fluconazole alone were resistant but the same strains were sensitive to low concentrations of TTO. The MIC values of fluconazole were ranged between 64- 256 μg/ ml
The MIC values of terpinen-4-ol ranged from 0.06% to 0.25% (average = 0.11 ± 0.09%); exposure of these strains for 24 hours to fluconazole and only 1/4 MIC of terpinen-4-ol strongly enhanced fluconazole activity against fluconazole-resistant C. albicans strains, the MIC values of terpinen-4-ol ranged from 0.06% to 0.25% (average = 0.11 ± 0.09%); exposure of these strains for 24 hours to fluconazole and only 1/4 MIC of terpinen-4-ol strongly enhanced fluconazole activity, and all of C. albicans isolates were classified as susceptible [63].

The anti-candidal activity of eugenol (main component of clove oil) and thymol (main component of thyme oil) either alone or in combination was evaluated against the architecture shape of the envelope of C. albicans. All investigated strains were susceptible to thymol and eugenol at MIC values of 125 μg/ ml and 500 μg/ ml respectively. Almost all of the untreated Candida cells were round or oval in shape with smooth surfaces, but exposure to eugenol or thymol induced a dramatic change in the morphology of the envelope. Also it was found that thymol proved to be about 40–50% more active than eugenol. On the other hand, the combination of 1 MIC of eugenol plus 1 MIC of thymol induced a significant increase in the number of damaged cells in comparison with the corresponding single concentrations of both molecules. The study concluded after measuring the MIC values of both molecules combined together in different incubation times, the presence of a synergistic effect [64].

A study was conducted to evaluate the antifungal activity of essential oil of Ocimum basilicum (Lamiaceae) leaves and its major components (i.e. linalool and geraniol), the species used in this study were both fluconazole-sensitive and resistant strains of Candida albicans and Cryptococcus neoformans. The results showed that all combinations produced FIC index values ranging from 0.3826 to 0.6326, also all these combinations significantly reduced their MIC values. The synergistic effect was observed in the combination of fluconazole and geraniol (MIC reduced from 31.25 to 4.14 μg/ ml and 76 to 19 μg/ ml respectively), and in the combination of linalool with geraniol (MIC values decreased from 790 to 111 μg/ ml and 76 to 19 μg/ ml respectively). It was also obviously seen that the concentrations needed from the two combined components to completely eradicate C. neoformans were very low. One interesting point was there was no synergistic effect in the combinations of natural components with fluconazole against C. albicans sensitive strain. However, a synergistic effect was observed in the combination of linalool with geraniol (MIC values reduced from 790 to 105 μg/ ml and 152 to 38 μg/ ml respectively). Furthermore, all combinations tested showed synergistic effect against C. albicans resistant strain. Fluconazole was also combined with the essential oil at concentration 156 μg/ ml, it was found that the MIC value was reduced from 500 to 1.01 μg/ ml. The combination of fluconazole with 197 μg/ ml linalool and 38 μg/ ml geraniol reduced its MIC value from 500 to 2.02 μg/ ml and to 1.04 μg/ ml respectively [65].

Essential oils of the dried parts of Origanum vulgare (Lamiaceae), Pelargonium graveolens (Geraniaceae) and Melaleuca alternifolia (Myrtaceae) were tested with nystatin against some Candida species. Micro dilution method was used to determine MIC and FIC values. The MIC of O. vulgare essential oil alone, MIC of O. vulgare essential oil with nystatin and the FIC of nystatin were ranged between 0.35–0.7 mg/ ml, 0.04–0.08 mg/ ml and 0.06–0.12 mg/ ml respectively. The MIC of one single sample and MIC of one sample of the most effective combinations and FIC for nystatin ranges between 2 and 8 mg/ ml; 0.1 and 0.4 mg/ ml; 0.02 and 0.05 mg/ ml respectively. The P. graveolens essential oil MIC of one single sample and MIC of one sample of the most effective combinations and FIC for nystatin ranges between 0.06 and 0.12 mg/ ml; 0.01 and 0.03 mg/ ml; 0.06 and 0.25 mg/ ml respectively. Few results were obtained to be additive (FICI = 40.5) for the associations nystatin with M. alternifolia essential oil. Also less effective results were obtained with P. graveolens and few results were additive effect (FICI = 40.5) for M. alternifolia essential oil. Also it has been shown that the nystatin-essential oil combination administered against the Candida species is likely to reduce the minimum efficient dose of nystatin. O. vulgare essential oil was the most effective among the essential oils. Some combinations of nystatin and P. graveolens essential oil did not have any synergistic effect for some of the strains considered. Associations of nystatin with M. alternifolia essential oil had only an additive effect [57].
Table 3. Some examples of a plant's essential oil combined with known antifungal agents; and the type of interactions which they cause

<table>
<thead>
<tr>
<th>Latin name of plant's essential oil used</th>
<th>Antifungal agent used</th>
<th>Fungal organism(s) used</th>
<th>Type of interaction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Melaleuca alternifolia</em></td>
<td>Clotrimazole</td>
<td>19 strains of <em>Malassezia pachydermatis</em> isolated from healthy dogs</td>
<td>Synergism</td>
<td>[56]</td>
</tr>
<tr>
<td><em>Mentha piperita</em></td>
<td>Clotrimazole</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Origanum vulgare</em></td>
<td>Clotrimazole</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Cinnamomum cassia</em></td>
<td>Clotrimazole</td>
<td>19 strains of <em>Malassezia pachydermatis</em> isolated from healthy dogs</td>
<td>Indifferent</td>
<td>[56]</td>
</tr>
<tr>
<td><em>Syzygium aromaticum</em></td>
<td>Clotrimazole</td>
<td></td>
<td></td>
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<tr>
<td><em>Melaleuca alternifolia</em></td>
<td>Clotrimazole</td>
<td>Reference strain of <em>Malassezia pachydermatis</em></td>
<td>Additive</td>
<td>[56]</td>
</tr>
<tr>
<td><em>Syzygium aromaticum</em></td>
<td>Nystatin</td>
<td>Some <em>Candida</em> species</td>
<td>Additive</td>
<td>[57]</td>
</tr>
<tr>
<td><em>Melaleuca alternifolia</em></td>
<td>Fluconazole</td>
<td><em>Candida albicans</em></td>
<td>Synergism</td>
<td>[58]</td>
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<td><em>Thymus broussonetii</em></td>
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<td><em>Thymus maroccanus</em></td>
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<tr>
<td><em>Agastache rugosa</em></td>
<td>Ketoconazole</td>
<td>Aspergillus <em>niger</em>, <em>A. flavus</em>, <em>Blastoschizomyces capitatus</em>, <em>Candida albicans</em>, <em>C. utilis</em>, <em>C. tropicalis</em>, <em>Cryptococcus neoformans</em>, <em>Trichoderma viride</em>, <em>Trichophyton tonsurans</em> and <em>Trichosporon mucoides</em></td>
<td>Synergism</td>
<td>[59]</td>
</tr>
<tr>
<td><em>Coriandrum sativum</em></td>
<td>Amphotericin B</td>
<td><em>Candida albicans</em></td>
<td>Synergism</td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Candida tropicalis</em></td>
<td>Additive</td>
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</table>
The essential oils of two aerial parts of Moroccan Thymus brousseonetti and T. maroccanus (Lamiaceae) with amphotericin B and fluconazole were tested against Candida albicans. Macro dilution broth method was used. Most of the essential oils showed significant anti-candidal activity with MIC of about 0.25 mg/ ml. Chekerboard titer assay was applied after combining the essential oils with either amphotericin B or fluconazole, the FICIs revealed significant decrease in the MIC values of the individuals, for example, the MIC of amphotericin B alone was lowered from 16 to 4 g/ ml in the presence of T. maroccanus essential oil. Synergistic effects were obtained after using different combinations of T. maroccanus and T. brousseonetti essential oils with either amphotericin B or fluconazole, as their FICI values were ranged between 0.27- 0.49. The results indicate that the synergistic effect of essential oils with fluconazole was stronger than the combination with amphotericin B. The study suggested that the use of these combinations are likely to reduce the minimum effective dose of the drugs, and hence minimizing their side effects and their treatment costs [58].

The anti-fungal activity of the essential oil of aerial parts of Agastache rugosa (Lamiaceae) and its main constituent estragole were investigated alone and their combinations with ketoconazole against 10 fungi using broth micro dilution, disk diffusion and checkerboard micro-titre assays. The 10 tested fungi were Aspergillus niger, A. flavus, Blastoschizomyces capitatus, Candida albicans, C. utilis, C. tropicalis, Cryptococcus neoformans, Trichoderma viride, Trichophyton tonsurans and Trichosporon mucoides. The MICs of the essential oil of A. rugosa were generally lower than the MIC value of estragole in most tested fungi. This finding suggests that the activity of the essential oil is probably based on estragole component, which makes up half of the essential oil content, while the other constituents have relatively mild activity. Ketoconazole had much higher activity than either estragole or A. rugosa essential oil with MIC values ranging between 12.5- 25 μg/ ml. When ketoconazole was combined with estragole it caused a significant decrease in the MIC value compared with each one alone. The isobologram constructed confirmed that ketoconazole– estragole combination is synergistic. The essential oil of A. rugosa showed a similar synergistic effect with ketoconazole producing an FIC index of 0.19 [59].

The anti-candidal effect of the essential oils of Satureja montana (Lamiaceae), Lavandula angustifolia (Lamiaceae), L. hybrida (Lamiaceae), Syzygium aromaticum (Myrtaceae), Origanum vulgare (Lamiaceae), Rosmarinus officinalis (Lamiaceae) and other chemotypes of Thymus vulgaris (Lamiaceae) on Candida albicans growth were studied individually. The essential oils of Thymus vulgaris thymol chemotype gave the strongest inhibitory effect (thymol content is 63.22% of the essential oil), and because of its promising activity it was studied in combination with amphotericin B; the results displayed that low concentrations from the essential oil produced a high increase in the MIC 80%, the strongest increase being obtained with a concentration of essential oil equal to 0.0025 μg/ ml. It can be noted that for concentrations ranging from 0.01 and 0.3 μg/ ml a linear decrease of the MIC 80% of amphotericin B was observed, if this decrease of the MIC 80% can be attributed to the anti-fungal action of the essential oil, it is difficult to explain the increase of the MIC 80% (i.e. antagonistic effect) observed with lesser quantities of the oil. Though the very weak concentration of the oil exhibit strong antagonism, synergism was observed with when concentration increased (concentration dependent). At concentrations of 0.2 and 0.3 μl/ ml the essential oil showed a decrease of the MIC 80% of amphotericin B compared with those of amphotericin B alone. The strongest decrease (48%) was achieved in medium containing 0.2 μl/ ml of the essential oil, while an essential oil concentration of 0.3 μl/ ml gave a total inhibition of the fungal growth with MIC 80% of amphotericin B equal to zero, therefore the presence of amphotericin B in the culture medium was not necessary. This study supports the potential role of essential oils from Thymus vulgaris thymol chemotype as an anti-fungal agent. The potentiation of amphotericin B exhibited by this essential oil may be promising for more effective and less toxic therapy for the treatment of mycoses [66].

The anti-fungal activity of the essential oil of Cinnamomum cassia (Lauraceae) either alone or combined with amphotericin B were investigated against C. albicans. The composition of the oil was analysed by GC/MS and showed high content of cinnamaldehyde (92.2%). Macro broth dilution method was applied to determine the MIC 80%. The results showed an increase of MIC values with essential oil concentrations ranging between 0.08- 0.5 μl/ ml and a decrease of MIC 80% was observed by comparison with
that of amphotericin B alone; the strongest decrease (70%) was obtained with a concentration of 0.1 μl/ml. This enhancement of amphotericin B activity may contribute for the development of less toxic and more effective therapies, especially in treatment of candidiasis associated with HIV infection [67].

Essential oils obtained from Cymbopogon martini (Poaceae) and Chenopodium ambrosioides (Amaranthaceae) leaves were tested for their anti-fungal activity, the oils were tested singly and in combination against dermatophytes and some filamentous fungi in vitro as well as in vivo by applying an ointment on a guinea pig model. The MIC of the essential oils (either individually and their combination) were compared for its effectiveness with the MIC of commonly used synthetic drugs (i.e. griseofulvin, ketoconazole and fluconazole). In in vitro study, both the essential oils, alone and their combination, displayed significant antifungal activity, the MIC values of the essential oil of C. martini against Microsporum gypseum and Trichophyton rubrum were 200 and 150 ppm respectively, they were comparatively less than the MIC values of the essential oil of C. ambrosioides against M. gypseum (700 ppm) and T. rubrum (350 ppm). After combination, the MIC values of both essential oils were also less than that of C. ambrosioides against M. gypseum (i.e. 500 ppm) and T. rubrum (i.e. 250 ppm). The MLC values of the essential oils and their combinations were ranged from 500 to > 1,000 ppm against the dermatophytes. T. rubrum was found to be the most sensitive against the essential oils. On the other hand, the MIC values of griseofulvin, ketoconazole and fluconazole were between 1,000- 5,500 ppm, which are much greater than the MICs of the essential oils and their combinations (i.e. 150–700 ppm). In in vivo study, the essential oil ointments were applied against induced ringworm in guinea pig model and disease removal was observed in 7–21 days, at day 5 of the treatment, randomly selected hairs of the inoculation areas were found to be positive for fungal culture on sabouraud dextrose agar; all the essential oil ointments were effective (C. martini > essential oils combinations > C. ambrosioides) in a time-dependent manner, the essential oil of C. martini showed complete cure of T. rubrum and M. gypseum infections at day 17 and day 21 respectively, while the essential oil of C. ambrosioides and its oil combinations cured the disease in most of the treatment models at day 21. The study concluded that the essential oils of both species are recommended in treatment of dermatophyte infections, and may applied as an alternative to synthetic drug for topical application because of their activity and synergism [68].

The antifungal activity of Coriandrum sativum (Apiaceae) essential oil either alone or its combination with amphotericin B was studied against two strains of Candida albicans and one strain of C. tropicalis by using micro dilution broth susceptibility assay and Checkerboard assay, respectively. The records represented that C. sativum essential oil has a fungicidal property with MLC values equal to the MIC value and ranging between 0.05- 0.4% (v/v), the fungicidal property was a result of cytoplasmic membrane damage and subsequent leakage of intracellular components such as DNA. Also, concentrations bellow MIC value caused obvious reduction in the percentage of germ tube formation in C. albicans strains. A synergetic effect was seen against C. albicans strains after applying C. sativum essential oil and amphotericin B together, while additive effect was seen against the essential oil of C. tropicalis[60].

The activities of essential oils from Allium sativum for. pekinense, A. cepa and A. fistulosum (Liliaceae) against three Trichophyton species(i.e. T. rubrum, T. erinacei and T. soudanense) were investigated and compared with the activity of allicin. The fungistatic activities of Allium species essential oils, allicin and ketoconazole among others were singly evaluated by broth dilution method and disk diffusion assay. From the results, A. sativum for. pekinense essential oil was the most potent inhibitor of all three Trichophyton species, with MIC values of about 64 mg/ ml, equivalent to 25– 50% of the activity of allicin (i.e. 16– 32 mg/ ml). The combinations of either A. sativum essential oil or allicin with ketoconazole were tested by the checker board titer test, the FICI values were ranged between 0.09- 0.12, it showed significant synergism of ketoconazole with Allium species. Moreover, the combination of Ketoconazole with allicin resulted in additive effects, with FICI values between 0.53 to 0.75[69].

The growth fungal inhibition of six herbal essential oils were tested against three Trichophyton spp (T. schoenleinii, T. erinacei and T. soudanense) alone and in combination with ketoconazole. Among the essential oil fractions tested alone, Cymbopogon citratus leaf (Poaceae) and Eucalyptus globulus leaf (Myrtaceae) were the most potent, with MIC
values of < 0.125– 0.25 mg/ ml and MFC values of < 0.125– 1 mg/ ml. Thymus vulgaris (Lamiaceae) essential oil and its main component thymol, gave high MIC values ranging between 0.25 and 1 mg/ ml, thymol was more active than the total essential oil of T. vulgaris. The essential oil of Pelargonium graveolens (Geraniaceae) as well as its main components (i.e. citronellol and geraniol) showed strong inhibition against these fungi, with MIC values between 0.25– 2 mg/ ml, and because of its strong effect, the combined effects between P. graveolens essential oil, citronellol and geraniol with ketoconazole were evaluated by using a checkerboard microtitre assay against Trichophyton spp, the MIC figures of ketoconazole when combined with P. graveolens essential oil were significantly lowered, with FIC indices ranging between 0.18 and 0.56. Moreover, in an experiment versus T. erinacei and T. soudanense, the FIC indexes of ketoconazole with citronellol or geraniol were 0.06 and 0.13 respectively. FIC indices indicate the strongest synergism between P. graveolens essential oil and ketoconazole against T. soudanense, with an FIC index of 0.18. Similar results were obtained by the combination of ketoconazole with geraniol or citronellol, with FIC index of again 0.18 [70].

Essential oils from Stems and leaves of 56 colombian plants [including Thymus vulgaris (Lamiaceae), Zingiber officinale (Zingiberaceae), Cunila origanoides (Lamiaceae), Eucalyptus citriodora (Myrtaceae), Morinda royoc (Rubiaceae), Lippia origanoides (Verbenaceae), Piper brede-meyeri (Piperaceae)] were assayed for anti-fungal activities, also they were tested in combination with either itraconazole or amphotericin B against clinical isolates of Candida albicans. The most active samples were the essential oils of P. brede-meyeri (MIC range 157.5– 222.7 µg/ ml), L. origanoides (MIC range 157.5– 198.4 µg/ ml) and M. royoc (MIC = 250 µg/ ml). The most synergistic effect was observed for the combination of itraconazole with essential oil of P. brede-meyeri (FICI range 0.09– 0.13), but no interactions were detected for the combination of amphotericin B with essential oil of P. brede-meyeri (FICI = 1.06) [71].

The aerial parts of ten medicinal plants [including Salvia officinalis (Lamiaceae), Pelargonium graveolens (Geraniaceae), Eucalyptus globules (Myrtaceae), Pistacia lentiscus (Anacardiaceae), Thymus capitatus (Lamiaceae), Nigella sativa (Ranunculaceae) seeds, Cinnamomum verum (Lauraceae) barks and Syzygium aromaticum (Myrtaceae) clove buds], among others were collected at their flowering stage and their essential oils were obtained by hydro-distillation method. The essential oils were investigated for anti-candidal activity and were evaluated for their potential synergism with fluconazole. Only C. verum, T. capitatus, S. aromaticum and P. graveolens essential oils showed broad spectrum activity against many pathogenic Candida strains. The synergistic property was exhibited with the combinations of C. verum fluconazole and P. graveolens/ fluconazole (FIC = 0.37), it was found that C. verum essential oil reduced the quantity of ergosterol to 83%, while P. graveolens essential oil may disturb the permeability barrier of the fungal cell wall, furthermore, the combinations with fluconazole causes disturbances in fatty acid homeostasis in cells of C. albicans as well as affecting their ergosterol biosynthesis, the quantity of ergosterol and oleic acid was reduced to 52.33% and 72% respectively [72].

3.4 Isolated Phytochemical(s) – Drug(s) Combinations

The following table (Table 4) shows some studies which had tested a combination of a plant’s isolated phytochemical compound with Known antifungal agents, and showed if there is any synergism, indifferent, additive or antagonism effects.

A study was conducted to evaluate some antifungal agents in combination with some monoterpene phenols, the phenoilic monoterpene compounds tested were carvacrol, thymol, eugenol and methyl eugenol, while the tested anti-fungal agents were fluconazole, amphotericin B, nystatin and caspofungin. 25 clinical isolates of Candida auris were involved in this study. MIC results showed that all tested compounds have anti-fungal activity at varying levels, carvacrol gave the best MIC value (125 µg/ ml) followed by thymol (MIC = 312 µg/ ml). The MFC values for the all four tested compounds were 1– 2 folds higher than their respective MIC values. Carvacrol was combined with fluconazole, amphotericin B, nystatin and caspofungin, the results showed both synergistic and additive effects in 68%, 64%, 96% and 28% respectively. Therefore the study recommended that carvacrol has a potential to be developed into a novel anti-fungal agent against C. auris [73].
Table 4. Some examples of a plant's isolated phytochemical compound combined with known antifungal agents; and the type of interactions which they cause

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<thead>
<tr>
<th>Name of isolated phytochemical compound used</th>
<th>Antifungal agent used</th>
<th>Fungal organism(s) used</th>
<th>Type of interaction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carvacrol</td>
<td>Fluconazole</td>
<td>25 clinical isolates of <em>Candida auris</em></td>
<td>Synergism</td>
<td>[73]</td>
</tr>
<tr>
<td></td>
<td>Amphotericin B</td>
<td></td>
<td>Additive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nystatin</td>
<td></td>
<td>Synergism</td>
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<td></td>
<td>Caspofungin</td>
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<td>Additive</td>
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<tr>
<td></td>
<td>Epigallocatechin gallate</td>
<td></td>
<td>Synergism</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td>Miconazole</td>
<td><em>Candida albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. kefyr and C. krusei</em></td>
<td></td>
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<td></td>
<td>Baicalein (flavones)</td>
<td></td>
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<td>[75]</td>
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<td></td>
<td>Amphotericin B</td>
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<tr>
<td></td>
<td>Fluconazole</td>
<td><em>C. parapsilosis</em></td>
<td>Synergism</td>
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</tr>
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<td></td>
<td>Osthole (coumarin)</td>
<td></td>
<td>Synergism</td>
<td>[76]</td>
</tr>
<tr>
<td></td>
<td>Eugenol, Methyleugenol</td>
<td></td>
<td>Synergism</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Magnolol (phenyl propanoid compounds)</td>
<td></td>
<td>Synergism</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td>Farnesol (monoterpene compound)</td>
<td></td>
<td>Synergism or additive</td>
<td></td>
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<td></td>
<td>Thymol</td>
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<td>[77]</td>
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<tr>
<td></td>
<td>Nystatin</td>
<td><em>Malassezia pachydermatis</em></td>
<td>Synergism</td>
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<td>Cinnamaldehyde</td>
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<td></td>
<td>Cinnamaldehyde</td>
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</table>

[37]
A research was conducted to determine the antifungal activity of curcumin obtained from *Curcuma longa* (Zingiberaceae) and to examine its possibilities to be combined with fluconazole and itraconazole. The MIC of fluconazole, itraconazole and curcumin was found to be in a range of 32-64 μg/ml, 8-32 μg/ml and 64-256 μg/ml respectively. The results of the in vitro anti-fungal study was based on measuring the zones of inhibition of the prepared combinations at standard concentration of 10 μg/ml, it was found that curcumin significantly increases the antifungal capacity of both fluconazole and itraconazole, and after calculating their FICls, it was found that the increase in anti-fungal capacity was due to either synergistic or additive effects. Furthermore, the topical sensitivity of the optimized combinations was determined by using rabbit vaginal model, and it was found to be free from any major signs of sensitivity [79].

Zwiebelane A, a Cyclic Organo-sulfur Compound from Onion (*Allium cepa*; Liliaceae) was tasted alone and in combination with Polymyxin B to evaluate its activities in fungal vacuole disruption against *Saccharomyces cerevisiae*. Zwiebelane A itself is ineffective against *S. cerevisiae* cells at 1.2 mM, whereas polymyxin B showed static activity at 60 μg/ml. The organism cells were subjected to lethal damage when polymyxin B was combined with zwiebelane A, while the normal architecture of the vacuoles was maintained when the organism cells were treated with either polymyxin B alone (60 μg/ml) or zwiebelane A alone (1.2 mM) [80].

Three saponins (i.e. ceposide A, ceposide B, and ceposide C) were isolated and extracted by acetone from the white onion bulbs, *Allium cepa* (Liliaceae), they were evaluated for their antimicrobial activity either alone or in selected combinations against ten fungal species, i.e. three soil-borne pathogens (*Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani* and *Sclerotium cepivorum*), five air-borne pathogens (*Alternaria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Mucor spp* and *Phomopsis spp*) and two antagonistic fungi (*Trichoderma atroviride* and *T. harzianum*). The results displayed that the antifungal activity of the three saponins increases related with their concentrations (i.e. ceposide B > ceposide A > ceposide C). When these saponins were combined, additive effects were detected, however, significant synergism effect was detected after applying mixture of 33.3% for each ceposide compound against *B. cinerea* and *T. atroviride*, the growth of these two fungi organisms was strongly inhibited when saponins were applied in combination with *B. cinerea* at 10 and 50 ppm [81].

Synergistic effects of tea catechin, epigallocatechin gallate (EGCG), alone and in combination with some common anti-mycotics against oral *Candida* spp was evaluated. The MIC of EGCG, miconazole, fluconazole and amphotericin B against biofilms of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. kafey* and *C. krusei* were determined by micro-dilution method. The results showed that EGCG inhibited *Candida* species growth at concentrations ranging between 375-1,500 μg/ml. However, it showed synergistic property against many *Candida* spp after combination with miconazole, fluconazole and amphotericin B (FICI range between 0.15-0.50). When EGCG was applied in combination with miconazole and amphotericin B, synergistic effect was seen against all species (The MICs of miconazole reduced from 0.25-1 to 0.031-0.25 mg/ml, The MICs of amphotericin B reduced from 0.063-0.25 to 0.016-0.063 mg/ml). For EGCG and fluconazole combination, however, no synergism was observed against *C. glabrata*, *C. krusei* and *C. kafey* [74].

Acteoside is an active compound which was obtained from the aerial parts of *Colebrookea oppositifolia* (Lamiaceae) which extracted by 50% hydro-ethanol. Acteoside and amphotericin B were examined each one alone and in combination against *Candida albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *Cryptococcus neoformans*, *Aspergillus flavus*, *A. fumigatus*, *A. niger* and *A. parasiticus* clinical isolates. The MIC values of amphotericin B were calculated in the absence and presence of increasing concentrations of acteoside (i.e. 0.195-12.5 μg/ml) by using two-dimensional checker board micro broth dilution method. The interesting finds is acteoside (alone) did not showed any anti-fungal activity at concentrations up to 1,000 μg/ml, but it showed synergistic property in combination with amphotericin B against all tested organisms (with FIC indices ranged between 0.0312-0.1562). The fungicidal effect of the combination of amphotericin B with acteoside was again assessed on *C. albicans*, *A. fumigatus* and *C. neoformans*, amphotericin B was used at a concentration of 0.256 x MIC (i.e. 0.1256 x MIC against *C. neoformans*), as well as in combination with increasing concentrations of acteoside ranging from 0.78 to 12.5 mg/ml, as expected, amphotericin B alone at these concentrations did not show any inhibitory action.
whilst the fungicidal activity (99.9% kill) was achieved at 46 x MIC (i.e. 26 x MIC against C. neoformans) after 24 hours when compared with the growth of the control. However, the same sub-inhibitory concentrations of amphotericin B resulted in fungicidal activity when tested in combination with acteoside at concentrations of 0.78–12.5 mg/ml for 24 hours. It’s to say the fungicidal activity of the combination was equivalent to the fungicidal activity of amphotericin B alone at 46 x MIC (26 x MIC for C. neoformans) because of the synergistic interaction [82].

In vitro anti-fungal activity of naturally occurring phenyl propanoids, eugenol and methyleugenol alone and in combinations against 64 fluconazole-sensitive and 34 fluconazole-resistant Candida clinical isolates was highlighted. FICI values were calculated from checkerboard micro dilution assays. The MIC values of eugenol and methyleugenol against C. albicans, C. tropicalis and C. glabrata were ranged between 475–500 mg/ml and 340–350 mg/ml respectively, while the MIC of fluconazole was ranged between 2.5–7.5 mg/ml and was within the reference ranges. It was noted that the resistant to fluconazole (i.e. MICs 80–110 mg/ml) showed sensitivity to both eugenol and methyleugenol. However, it is clear that methyleugenol proved to be more active against all Candida species than eugenol. The anti-fungal activity of both compounds was increased with increasing of their concentrations. All the fluconazole-susceptible and resistant Candida isolates showed high degree of sensitivity from their large inhibition zones. The FICI values for eugenol/fluconazole and methyleugenol/fluconazole combinations against all fluconazole-sensitive isolates were ranged between 0.31–0.55 and 0.24–0.58 respectively, the interaction of eugenol/fluconazole combination was synergistic in 58 out from 64 isolates, whereas for methyleugenol/fluconazole it was 59, then only 5 isolates showed indifference property. Out of 34 fluconazole-resistant strains, 29 and 31 isolates showed synergistic affects for eugenol/fluconazole and methyleugenol/fluconazole respectively. No antagonistic activity was seen in this study. The study recommended that fluconazole can be supplemented with eugenol and methyleugenol to treat fluconazole-resistant Candidiasis [83].

Evaluation of the in vitro activity of baicalein, a flavone constituent of Scutellaria baicalensis (Lamiaceae), and its combination with fluconazole against Candida albicans, C. tropicalis and C. parapsilosis was conducted. The MIC50 of baicalein alone ranged from 13 to 104 mg/ml. Exposure to baicalein at MIC50 values obtained for each strain and at 260 mg/ml resulted in significant anti-candidal activity. The anti-fungal activity of fluconazole/ baicalein combination was greater than the individual contribution of each agent, according to FICI values, the combination showed partial synergistic property against C. albicans and C. tropicalis, while the combination produced synergistic property against C. parapsilosis [75].

The activity of catechins isolated from green tea leaves of Assam Camellia sinensis (Theaceae) were tested alone and in combinations with fluconazole, amphotericin B and copper sulphate against some Candida spp following micro-dilution checkerboard technique and time kill assay. The MIC50 of the purified catechins against C. albicans was observed at 125 mg/ml, while its minimum fungicidal activity (MFC) was shown between 250–1 mg/ml. The MIC of Fluconazole was observed at concentrations of 64 mg/ml and 128 mg/ml for C. albicans and C. glabrata respectively, while the MIC value of Amphotericin B was observed at concentrations of 1 mg/ml against both Candida species. Catechins showed synergistic effect with fluconazole and amphotericin B against Candida spp. Time kill assay also showed synergistic effect at MIC and twice of the MIC of purified catechins and it’s combinations. Furthermore, copper sulphate increased the anti-candidal activity of the synergistic combinations by 0.4% to 6.63%. The study concluded that the promising anti-candidal activity requires further investigations of safety profile for green tea based potent therapeutic drug [84].

The in vitro anti-fungal effects of osthole, a natural coumarin compound derived from Cnidium plant (Apiaceae), was investigated alone and in combination with fluconazole against Fluconazole-resistant Candida albicans. A total of 30 clinical fluconazole-resistant C. albicans isolates were applied (MIC50 greater
than or equal to 8 μg/ ml), and 10 fluconazole-susceptible C. albicans isolates (MIC≤20 less than or equal 1 μg/ ml) were also involved in this study. The results showed that osthole alone did not showed anti-fungal activity (i.e. MIC≤20 was greater than 64 μg/ ml). The combination of osthole with fluconazole showed significant synergistic effect against the fluconazole-resistant C. albicans, the dose of fluconazole was reduced from 1 to 16 μg/ ml, and the dose of osthole was reduced from 4 to 16 μg/ ml, also the FICI 0.04 to 0.31, the synergistic effect was dose-dependent according to the growth curve assay. Unlike the result report of fluconazole-resistant isolates, both fluconazole and osthole did not displayed synergistic effect on fluconazole-susceptible strains, as the FICI was 0.51 to 2.01 [76].

Anti-fungal activity of cinnamaldehyde, eugenol, honokiol, magnolol and shikonin was evaluated, either alone or in combination with fluconazole against some Candida species. When the phytochemicals were tested alone, some exhibited significant anti-fungal activity, with MICs of ≤8 μg/ ml, and other compounds were even more potent than fluconazole or itraconazole (i.e. honokiol, magnolol and shikonin). In the group of phenylpropanoids, some compounds demonstrated slight or moderate efficacy, such as cinnamaldehyde, eugenol and magnolol. However, in combination with fluconazole they showed significant synergistic effects, including resistant strains of Candida species. For instance, when eugenol, methyleugenol and magnolol were used in combinations with fluconazole, the FICI values revealed high synergism (FICI<0.5). On the other hand, some terpenoids like farnesol were tested, it showed synergistic and additive effects with fluconazole against drug-resistant Candida isolates and C. albicans biofilms [77].

Anti-candidal activity of two asarones (α and β) isolated from alcoholic extract of Acorus calamus (Acoraceae), were tested in combinations with either fluconazole, clotrimazole or amphotericin B, the tested organisms were Candida albicans and C. tropicalis. The highest activity was shown by β-asarone, with MIC values ranged between 64–125 μg/ ml, while α-asarone showed the activity at MIC values ranged between 250–500 μg/ ml, for azole drugs the activity was ranged between 1–4 μg/ ml and for amphotericin B the activity was ranged only between 1–2 μg/ ml. The combined anti-candidal activities of asarones and the chosen drugs were assessed by using checkerboard micro-dilution and time-kill assays, the results displayed significant synergistic effect, especially the combinations of β-asarone with azoles and amphotericin B. Antagonism and indifference effects were not recorded in all combinations, the MIC values have been reduced to more than 8 times in the combinations of α and β asarones with azoles and amphotericin B [85].

β carbolines such as harman, harmine, harmaline and harmalol are pharmacologically active alkaloids which are present in the seeds of Peganum harmala (Nitriariaceae). These β-carboline alkaloids are extracted by methanol through bioassay-guided fractionation process and their anti-fungal activities were investigated either alone or in combinations against Aspergillus niger and Candida albicans. The isolated β-carboline alkaloids showed antimicrobial effects against all tested microorganisms as the diameters of zone of inhibition were ranged between 10.5 and 31.5 mm. When the alkaloids were examined individually, C. albicans was the most susceptible to harmine (22.2 mm), while harman was the most active against A. niger (20.8 mm). Harmaline was more effective against C. albicans (21.3 mm), meanwhile, harmalol showed moderate activities. A combination of harman and harmaline mixture was active against C. albicans (29 mm). The lowest minimal value of 0.333 mg/ ml was recorded with the total (crude) harmala alkaloids, and the mixtures of harman with either harmine or harmaline [86].

The in vitro anti-fungal activity of carvacrol, cinnamaldehyde and thymol, alone and in combinations with fluconazole, itraconazole, ketoconazole, clotrimazole, miconazole, terbinafine and nystatin against Malassezia pachydermatis was investigated. The combination results showed the presence of synergism, indifference and antagonism, based on MIC values, the highest synergistic interaction (80%) was seen in the following combinations: thymol + nystatin, carvacrol + nystatin and carvacrol + miconazole, the other combinations produced synergistic interactions that were ranged between 16.6% to 70%. The highest indifference interaction (70%) was seen in the combinations of cinnamaldehyde + fluconazole, thymol + terbinafine and cinnamaldehyde + terbinafine. The highest antagonistic interaction was formed from the combinations of carvacrol + ketoconazole, thymol + ketoconazole (40%) and cinnamaldehyde + ketoconazole (46.6%) [78].
Hydrated catechin, hydrated quercetin and (-) epigallocatechin gallate were combined with fluconazole and examined against fluconazole-resistant Candida tropicalis strains. All strains had showed MIC\textsubscript{50} value of 64 µg/ ml for fluconazole. The flavonoids when tested alone had not shown any anti-fungal activity, but when they were used as a co-treatment with fluconazole, there was significant synergistic effect. The synergism between the flavonoids and fluconazole was determined by using checkerboard technique, the FICI values were ranged between 0.25- 0.38 µg/ ml [87].

3.5 Plant Latex – Drug(s) Combinations

The following table (Table 5) shows studies which had tested a combination of a plant’s latex exudate with Known antifungal agents, and showed a synergism effect.

Euphorbia characias (Euphorbiaceae) latex production was initiated after making repeated cuts along the stems, and had been collected by Eppendorff tubes and stored at 4 °C. The in vitro susceptibility of Candida albicans to ketoconazole and E. characias latex either alone for each or in combination of both was examined using macro-broth dilution method. The concentration of latex was estimated by its proteins content via Bradford’s method. The MIC 80% of the crude latex and ketoconazole were 159 µg protein/ ml and 0.3901 µg/ ml respectively. The examination of the mixture containing latex at several concentrations (i.e. 7.8, 15.62, 31.25, 62.5 and 125 µg protein/ ml) combined with ketoconazole indicates a synergistic effect; in the case of latex, after its concentrations of 31.25 and 62.5 µg protein/ ml were tested, it was found that the MIC 80% of ketoconazole were lowered to 0.194 and 0.183 µg/ ml respectively, as the MIC 80% of ketoconazole alone was 0.390 µg/ ml [88].

Carica papaya (Caricaceae) latex sap (0.41 mg protein/ ml) in combination with 2 µg/ ml of fluconazole were examined against the growth of Candida albicans. The mixture showed synergistic action, this synergistic effect was due to partial cell wall degradation. When the concentration of fluconazole was increased to 4 µg/ ml, it was recorded that there is a small decrease of MIC 80% of the latex (i.e. from 150 to 130 µg protein/ ml) [89].

The Hevea brasiliensis (Euphorbiaceae) latex obtained from the rubber trees was treated with ammonia (to prevent rubber coagulation), amphotericin B was dissolved in 100% DMSO. The anti-fungal activity of the latex was tested with various fungal strains by using macro-broth dilution assays, also the MIC 80% was estimated singly and in combination with amphotericin B. In individual assay, it was concluded that the growth of all tested organisms was inhibited by H. brasiliensis latex, the strongest antifungal activity was achieved against Trichosporon cutaneum (i.e. MIC 80% = 40.615 µg protein/ ml) and Cryptococcus neoformans (i.e. MIC 80% = 56.078 µg protein/ ml). In the combination assay, Candida albicans was cultured on a medium supplemented with different concentrations of H. brasiliensis latex and amphotericin B combinations, the concentration of the latex was ranged between 7.5 to 60 µg protein/ ml, it was found that the best MIC 80% (0.201 µg amphotericin B/ ml) was observed when the culture medium contained 60 µg protein/ ml, the use of 15 and 30 µg protein/ ml gave MIC values that were slightly higher (0.221 and 0.247 µg protein/ ml respectively), also a great MIC 80% (0.369 µg amphotericin B) was observed with the addition of 7.5 µg protein/ ml in the culture medium. The study concluded that the MIC 80% decreases strongly for the latex concentrations in a range between 0– 15 µg protein/ ml, then decreases very slightly with higher concentrations up to 60 µg protein/ ml, amphotericin B showed synergistic effect with all tested H. brasiliensis latex concentrations, the rates of synergy were about 50, 44 and 55% with 15, 30 and 60 µg protein/ ml latex respectively [90].

Table 5. Examples of a plant’s latex exudate combined with known antifungal agents; which showed synergism effect

<table>
<thead>
<tr>
<th>Latin name of plant’s latex used</th>
<th>Antifungal agent used</th>
<th>Fungal organism used</th>
<th>Type of interaction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euphorbia characias latex</td>
<td>Ketoconazole</td>
<td>Candida albicans</td>
<td>Synergism</td>
<td>[88]</td>
</tr>
<tr>
<td>Carica papaya latex sap</td>
<td>Fluconazole</td>
<td>Candida albicans</td>
<td>Synergism</td>
<td>[89]</td>
</tr>
<tr>
<td>Hevea brasiliensis sap</td>
<td>Amphotericin B</td>
<td>Candida albicans</td>
<td>Synergism</td>
<td>[90]</td>
</tr>
</tbody>
</table>
4. CONCLUSION
To conclude, many people are using combination therapy in treatment of many fungal infections, specially in developing countries, these combinations are either plant-plant combinations or plant product-antifungal drug combinations, many of these combinations are used in the practice of traditional medicine from ancient time (specially plant-plant combinations), these combinations lead to herbal-herbal or herbal-drug interactions, in which these interactions can be useful (synergistic effect) or may lead to a decrease in the overall antifungal activity (i.e. antagonistic effect). That is why many researches had be conducted to test and to prove the efficacy and safety of many combination preparations used as anti-fungal agent in traditional medicine, so the main aim of this review was to collect many researches which had tested all of these combinations, and listed them in a form of synergistic, additive, indifferent or antagonistic effects, this will help many researchers to choose a combination preparations which have synergistic effect for further analysis, and to avoid preparations which have antagonistic effect. Moreover, our review showed that combinations with synergistic effect will offer significant opportunity to develop novel antifungal therapies, specially against resistant fungal organisms, this will decrease the opportunity to develop resistance and also reduce adverse effects for some current available antifungal agents. We recommend that further studies are needed to study the pharmacokinetic and pharmacodynamic profiles of these novel synergistic combinations.

CONSENT
It is not applicable.

ETHICAL APPROVAL
It is not applicable.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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