IN-VITRO ACTIVITIES OF SELECTED MEDICINAL PLANT EXTRACTS AGAINST POST HARVEST PATHOGENIC FUNGI

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Abstract
Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against fungi. Fungi can often cause severe diseases in vegetable and fruit species. This study is investigate the potential antifungal activities of traditional medicinal plants currently used in the community for the treatment of fungal infection in post-harvest fruit and vegetables. Fresh clean leaves of *Datura stramonium* (80gm), fresh clean root bark of *Croton macrostachyus* (95gm) and fresh clean leave of *Laggera tomentosa* (110gm) were soaked in conical flask containing 500 ml petroleum ether solvent. A total of three etiological agents of post-harvest fruit and vegetable decay, *Penicillium italicum*, *Penicillium expansum*, and *Phytophthora citrophthora* were taken to evaluate the antifungal activity of plant extracts. *Datura stramonium* extracted have been provided the highest amount of crude yield from 80gm initial samples extracted by chloroform (16.25%) and the lost amount of crude extracts recorded in *Laggera tomentosa* from initial sample of 110 gm extracted by petroleum ether and acetone (2.73%). *Datura stramonium* chlorofrm extract and *Datura stramonium* acetone extract have highest activity against *Penicillium expansum* with zone of inhibition 15.6±1.5 and 14±0 respectively. Furthermore *Datura stramonium* chlorofrm extract has best activity against *Phytophthora citrophthora* with zone of inhibition 13.6±0.3. Only flavonds was found in the three medicinal plants and *Laggera tomentosa* was tested positive for four type phytochemicals such as alkaloids, tannins, flavonds and glycosides. The activities observed could be due to the presence of some of the secondary metabolites like, alkaloids, tannins, sterols, glycosides, saponins, terpenes and flavonoids present in the plant. Hence, further high performance liquid chromatography and mass spectroscopy analysis should be better in order to identify to which active compound related to fungicidal power and to know the structure of respective compound.

Keywords: Medicinal plant; Pathogenic fungi; Vegetable and fruit; Crude extracts.

1. Introduction

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against fungi. Considerable economic damages to plant products have been recorded. Among them, fungi constitute the more numerous group of plant pathogens and can often cause severe diseases in vegetable and fruit species (Chang et al. 2008). Over several decades, various attempts have been accomplished to prevent, control, or eradicate plant diseases, and development of synthetic fungicides was particularly investigated (Lee et al. 2009). These pesticides are known to be highly effective in controlling various postharvest diseases of vegetables and fruits. Although effective, their continued or repeated applications may disrupt equilibrium of ecosystems, leading to dramatic disease outbreaks, widespread development of pathogens resistant to one or more chemicals, toxicity to non-target organisms and environmental problems (Lee et al. 2009). Sometimes, they accumulate in the food chain as residues above safe limits (Lee et al. 2008). Furthermore, pesticide residues in food possess more carcinogenic risks than insecticides and herbicides (Lee et al. 2009).

Studies on plant-derived fungicides is now being intensified, as it became evident that these substances have enormous potential to improve the future agrochemical technology. In fact, there are good reasons to suppose that secondary plant metabolism has naturally evolved to actively protect vegetable and fruit species from microbial pathogen attacks (Kim et al. 2003). Since secondary plant metabolites are often active against a small number of specific target microorganism species and are biodegradable to nontoxic products, they are potentially useful in integrated pest management programs: moreover, they could allow developing a new class of possibly safer disease control substances. Therefore, efforts have been focused on secondary plant metabolites for their potential use as commercial fungicides or as lead compounds (Lee et al. 2001).

Synthetic fungicide is currently used as primary means for the control of plant disease. However, the alternative control methods are needed because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicide among fungal pathogens, and high development cost of new chemicals. The uses of plant-derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance (Lee et al. 2007). Essential oils are concentrated, hydrophobic liquid containing volatile aromatic compounds extracted from plants (Isman, 2000). They were previously to have biological activities such as antifungal (Soliman & Badeaa, 2002), antibacterial (Dorman et al. 2000), insecticidal and nematicidal effects (Pandey et al. 2000).

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to nutritional value, organoleptic characteristics, and limited shelf life (Agrios, 2004). In addition, in some cases fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens. The fungal decay of fruits and vegetables in postharvest storage greatly limits their economic value. Although fungicides treatments have been the main method for controlling postharvest diseases, public concern about fungicides residues in food and the development of fungicides resistance by pathogens has increased the search for alternative means of controlling the disease. Biological control of postharvest decays of fruits and vegetables has emerged recently as a promising alternative to the use of synthetic fungicides (Wisniewski & Wilson, 1992).

Growing and marketing of fresh fruit in Ethiopia are complicated by post-harvest losses both in terms of quality and quantity between harvest and consumption. The quality of fresh fruit depends up on the harvesting activities, post-harvest handling, transportation and storage (Haider & Demisse, 1999; Asmaru et al. 2013). In response to the increasing food borne illness, governments all over the world are intensifying their effort to improve fruit safety. However in Ethiopia no sufficient continuous survey or assessment of fruit safety has been developed. The researcher has been motivated to fill the gap. Therefore, this study has tried to determine the management of fruits in Dessie and Kombolcha town. In our country, many of the research works emphasize on the check-list form of the selected medicinal plants rather evaluation of the potential plant extracts and phytochemical compounds. This study is, therefore, initiated to bridge such knowledge gap by evaluating antimicrobial activity of the crude extracts of some of the commonly used medicinal plants against common fungi pathogens.

3. Materials and methods

3.1. Gathering of potential medicinal plant

A total of three medicinal plants were collected based on their important for combating fruit and vegetable fungal pathogens according to literature, national herbarium data and information obtained from local inhabitants during ethno-medicinal survey conducted in this study. Hence structured questionnaires was administer systematically in and around Dessie and Kombolcha town to mothers, medicinal plant sellers, community leaders and elders in the community to obtain information on commonly predominant fungal disease therapy. Accordingly these plants are; *Datura stramonium* (Astenagr), *Croton macrostachyus* (Bisana) and *Laggera tomentosa* (Alashume). The taxonomic identities of these plants were confirmed by botanist or taxonomist in Addis Ababa National herbarium.

3. 2. Extraction of plant material via maceration (soaking)

Fresh clean leaves of *Datura stramonium* (80gm), fresh clean root bark *Croton macrostachyus* (95gm) and fresh clean leave *Laggera tomentosa* (110gm) were soaked in conical flask containing 500 ml petroleum ether solvent. The soaked leaves was stayed for 72 hours with shaking of the extracts in the intermediate time. After 3 days the extract was filtered by using Vucher separatory funnel and the collected

extract was further separated by rotary evaporator at 40 ^{o}C reduced temperature. Finally the crude extract was placed in desiccators containing $CaCl_2$. The dried extract was put in refrigerator for further uses. In the second gradient extraction step 500 ml chloroform was added and soaked for 72 hours filtered by Vucher separatory funnel and finally 500 ml of acetone was added in the conical flask containing sample and filtered by the same manner like the above.

3.3. Culture media used

Potato dextrose agar and sabouraud agar (SDA) were used during the study. Sabouraud agar (SDA) was used for the antimicrobial tests and for determination of minimum inhibitory concentration; more over potato dextrose agar was also used for routine stock cultures and sub culturing.

3.4. Test fungal isolates (strains)

A total of three etiological agents of post-harvest fruit and vegetable decay, *Penicillium italicum*, *P. expansum*, and *Phytophthora citrophthora* were taken to evaluate the antifungal activity of plant extracts. The test microorganisms were obtained from Pasteur Institute, Addis Ababa and the rest of the isolates was screened from samples brought from Kombolcha and Dessie town. The standard strains and isolates was cultivated and kept on potato dextrose agar (PDA) at 4 °C for further activities.

3.5. Antifungal assays:

3.5.1. Agar well diffusion assay

Preliminary analysis of antifungal activity was conducted using agar well diffusion assay as described by Smania et al. (1995). The Fungal inoculums were prepared in Tween 80 saline solution. Each fungal suspension was poured into the sterilized petriplates. After that molten sabouraud agar (SDA) medium was poured into the petriplates containing inoculum and rotated to mix the inoculum and the medium uniformly and kept for solidification. After solidification wells of 6 mm diameter were bored with the help of sterilized borer. The wells were filled with different concentration of plant extracts. Twenty percent DMSO were used as a solvent to dissolve the plant extracts. Amphotericin B, Fluconazole, Clotrimazole and Nystatin were used as positive controls. The plates were incubated at 25 ^{o}C for 2-3 days. The results were also express in terms of the diameter of the inhibition zone. All experiments were carried out in triplicates.

3.5.2. Determination of the minimum fungicidal concentration

The minimum fungicidal concentration (MFC) of the selected plant extracts was determined according to a standard procedure as described by Espinel-Ingroff et al. (2002). Following an overnight incubation for the MIC determination, different concentrations of the extracts was diluted and incubated at optimum temperature for 24 to 48 h until the visible growth was observed. The MFC value was the concentration where no growth or fewer than three colonies were obtained to give approximately 99 to 99.5% killing activity.

3.6. Phytochemical analysis

The most common Phytochemicals (secondary metabolites) such as alkaloids, glycosides, flavonoids, tannins and saponins present in powdered forms of the study three medicinal plants were analyzed following methods described in Rasool et al. (2010).

3.6.1. Test for tannins

Half gram of the powdered plant materials were boiled in 10 ml of distilled water in a 100 ml beaker sized and then filtered; few drops of 0.1% ferric chloride (FeCl3) were added. Formation of brownish green or a blue-black coloration indicates the presence of tannins (Ayoola et al., 2008).

3.6.2. Test for alkaloids

From about 0.5 g of powdered plant materials boiled in 10 ml of prepared acid alcohol and filtered, about 5 ml of the filtrate was taken and 2 ml of dilute ammonia added. Then 5 ml of chloroform was also added and shaken gently. The chloroform layer was extracted with 10 ml of acetic acid. Formation of a cream with Mayer's reagent confirms the presence of alkaloids (Ayoola et al. 2008).

3.6.3. Test for saponins

To 0.5 g of powdered plant materials in a test tube, 5 ml of distilled water was added and the mixture was vigorously shaken. Formation of a froth Persistent for 30 min confirms the presence of saponins (Ayoola et al. 2008).

3.6.4. Test for flavonoids

To a portion of an aqueous filtrate of the powdered plant materials about 5 ml of dilute ammonia solution was added. Concentrated sulphuric acid (1 ml) addition and yellow colorations that disappeared on standing indicated the presence of flavonoids (Ayoola et al. 2008).

3.6.5. Test for glycosides

To 2 ml alcoholic filtrate plant materials, 1 ml glacial acetic acid and 1-2 drops of $FeCl_3$ was added and 1 ml of concentrated H_2SO_4 followed. Appearance of brown ring at the interface indicated presence of cardiac glycosides. A violet ring also appeared below the brown ring confirm positive for cardiac glycosides (Trease and Evans, 1989).

3.8. Data analysis

During analysis; means and standard deviation of the replicates test of the antifungal activities of the crude plant extracts were calculated. Statistical differences was considered at P-value less than 0.05 ($P<0.05$).

4. Results

4.1. Background information of the study population

Preliminary survey were conducted for understanding of the local inhabitants perceptions towards traditional medicinal plants, before conducting the plant crude extraction. For these purpose, a total of 120 interviewee participated in provision of information pertaining to the use of medicinal plants and all the participants were aged ≥ 30 years. Of the total interviewees, 35 (29.17%) were males and the rest 85 (70.83%) were females. Of the respondents, 118 (98.33%) were non-herbalist and the remaining 2 (1.67%) were local herbalist. The analysis of gathered information showed that the people have been using traditional medicinal plants for treatment of different type of disease including *Malassezia furfur and* decaying of fruit. According to the respondents' opinion against fungal disease, the degree of importance of the traditional medicinal plants were ranked as follows: 46% of *Datura stramonium* (Astenagir), 29% *Croton macrostachyus* (Bisana), 11.35% *Laggera tomentosa* (Alashume) and others 13.65%. During survey results of the study implied that 85% of the inhabitants are practicing the leave parts of the folkloric medicinal plants and the less accounts like the stem, bark, root and combination of its parts according to the type of plant used.

Table 1. Profile of the studied medicinal plants.

No.	Name of Medicinal Plants	Vernacular Name	Family	Plant Part Used	Traditional Uses	Place of Collection
1	*Datura stramonium*	Astenagr	Solanaceae	Leave	*Malassezia furfur*	Dessie
2	*Croton macrostachyus*	Bisana	Euphorbiaceae	Root bark	Stomach Ache and *Malassezia furfur*	Kombolcha
3	*Laggera tomentosa*	Alashume	Asteraceae	Leave	anti-inflammatory like tinea nigra	Near to Dessie

4.2. Crude extract yields of medicinal plants

Two selected medicinal plant leaves and one selected medicinal plant root bark were extracted via petroleum ether, chloroform and acetone (table 2). The obtained crude extracts were calculated in percentage yields. Based on the result *Datura stramonium* extracted have been provided the highest amount of crude yield from 80gm initial samples extracted by chloroform (16.25%) and the lost amount of crude extracts recorded in *Laggera tomentosa* from initial sample of 110 gm extracted by petroleum ether and acetone (2.73%).

Table 2. Crude extraction yield of the three studied medicinal plants in different solvents

No.	Plants Name	Solvents	Initial Sample Amount (gm)	Extracted Yield (mg)	Percentage Yield (%)
		P. ether	80	11	13.75

1	*Datura stramonium*	Chlorofrm	80	13	16.25
		Acetone	80	9	11.25
2	*Croton macrostachyus*	P. ether	95	7	7.37
		Chlorofrm	95	8	8.42
		Acetone	95	7	7.37
3	*Laggera tomentosa*	P. ether	110	3	2.73
		Chlorofrm	110	5	4.55
		Acetone	110	3	2.73

4.3. Antifungal activity of medicinal plants

Selected medicinal plants were extracted via soaking method of extraction and antifungal activities were determined. The tests were conducted in triplicates and results put in mean values of the triplicate tests. Accordingly, mean antifungal activity values of the plant extracts (at concentration of 100 mg/ml) are as show in Table 3. *Datura stramonium* chlorofrm extract (DSC) and *Datura stramonium* acetone extract (DSA) have highest activity against *Penicillium expansum* with zone of inhibition 15.6±1.5 and 14±0 respectively. Furthermore, *Datura stramonium* chlorofrm extract (DSC) has best activity against *Phytophthora citrophthora* with zone of inhibition 13.6±0.3. In addition to *Datura stramonium* plant, the *Croton macrostachyus* choloroform extract (CMC) and *Croton macrostachyus* petroleum ether extract (CMP) have showed second best active against the *Penicillium italicum* with zone of inhibition 11.3±0.57 and 10±1 respectively.

Table 3. Antifungal activities of selected medicinal plants extracted by soaking method (concentration, 100 mg/ml).

Name of extracts	*Fungal species*		
	Penicillium italicum	*Penicillium expansum*	*Phytophthora citrophthora*
DSP	12.3±0.57	7.5±0.1	NA
DSC	9.3±0.47	15.6±1.5	13.6±0.3
DSA	12±1	14±0	NA
CMP	10±1	8.6±0.5	5±1
CMC	11.3±0.57	3.6±0.57	NA
CMA	7.6±2	NA	NA
LTP	3.3±0.57	4.6±1	NA
LTC	4±0	NA	2.5±0.56
LTA	6.3±0.57	NA	NA

As showed in table (3), there are many acronyms written in abbreviation, the extended words are; DSP= Datura stramonium Petroleum ether extract, DSC= Datura stramonium Choloroform extract, DSA= Datura stramonium Acetone extract, CMP = Croton macrostachyus Petroleum ether extract, CMC= Croton macrostachyus Choloroform extract, CMA= Croton macrostachyus Acetone extract, LTP = Laggera tomentosa Petroleum ether extract, LTC= Laggera tomentosa Choloroform extract, LTA= Laggera tomentosa Acetone extract and NA=do not have activity, Values are mean inhibition zone (mm) ± S.D of the replicates.

A total of five extracts were selected for determining the minimum inhibitory concentrations (MIC) (table 4). The selection was based on the potent preliminary activity test. Based on the above reason, DSP, DSC, DSA, CMP and CMC were evaluated in the concentration range 100 mg/ml to 1.75 mg/ml (70 mg/ml, 50 mg/ml, 30 mg/ml, 15 mg/ml, 7.5 mg/ml, 3.5 mg/ml, and 1.75 mg/ml). The fungal species were highly susceptible by Datura *stramonium* that means from 30-3.5 mg ranges of concentration.

Table 4. Minimum inhibitory concentrations of selected extracts

Fungal species	DSP	DSC	DSA	CMP	CMC
Penicillium italicum	15	7.5	30	15	70
Penicillium expansum	7.5	3.5	30	50	100

Phytophthora citrophthora	NA	3.5	NA	100	NA

4.4. Phytochemical screening

All the evaluated medicinal plants have been tested for the presence of alkaloids, tannins, saponins, flavonds and glycosides. Only flavonds was found in the three medicinal plants and *Laggera tomentosa* was tested positive for four type phytochemicals such as alkaloids, tannins, flavonds and glycosides as showed in table 5.

Table 5. Phytochemical constituent of selected medicinal plants

Tests for	**Name of Plants**		
	Datura stramonium	*Croton macrostachyus*	*Laggera tomentosa*
Alkaloids	+	-	+
Tannins	-	+	+
Saponins	-	+	-
Flavonoids	+	+	+
Glycosides	+	-	+

Where, the (-) indicate absence and the (+) indicate presence

5. Discussion

5.1. Background information of the study population

Three traditional medicinal plants were considered for investigation based on literature and the local inhabitants' preference for the treatment of fruits and vegetable disease. Accordingly, *Datura stramonium* (Astenagr), *Croton macrostachyus* (Bisana), *Laggera tomentosa* (Alashume), were the most commonly used traditional medicinal plants. Response of most of the interviewee indicated that leaf parts were the frequently used part of traditional medicinal but some plants are part restricted to treat the fungal disease. All of the respondents have the opinion that using of the traditional medicinal plants for the treatment of *Malassezia furfur* on human as well as eating of the treated fruit and vegetable has no side effects; probably the dilutions of plant materials during used reduce the toxic bioactive molecules. However, literature evidence shows that some medicinal plants have side effects; as the occurrence of heavy metals like lead, mercury, arsenic and cadmium may decline medicinal value of homeopathic plants (WHO, 2009).

5.2. Evaluation of antifungal activities of crude extracts

All crude extracts of three medicinal plants obtained from soaking methods were evaluated for antifungal activity. Based on the present results, *Datura stramonium* chlorofrm extract (DSC) and *Datura stramonium* acetone extract (DSA) have highest activity against *Penicillium expansum* with zone of inhibition 15.6±1.5 and 14±0 respectively. Furthermore, *Datura stramonium* chlorofrm extract (DSC) has best activity against *Phytophthora citrophthora* with zone of inhibition 13.6±0.3. In addition to these, the *Croton macrostachyus* choloroform extract (CMC) and *Croton macrostachyus* petroleum ether extract (CMP) have showed second best active against the *Penicillium italicum* with zone of inhibition 11.3±0.57 and 10±1 respectively. The study conducted by Egharevba et al. (2010) reported that the *in vitro* antimicrobial screening revealed that the extract exhibited varying activity against different microbes with zones of inhibition ranging from 14-32 mm. Some of the results were in agreement with the current study and some of them were highly potent than the current study. The degree of antifungal activity influenced by the suitable solvents used for extraction; it could also depend up on the condition or state of the sample along with the season in which the plant was collected. Several factors such as age of the plant, duration of storage, temperature, pH, preparation of media could also directly or indirectly affect the activities of extracts on the fungal species (Rao, 1995). On the other hand the study conducted by Udegbunam et al. (2015) reported that the extract exhibited in-vitro antimicrobial effect in a concentration-dependent manner with one hundred (100) mg/ml concentration of the extract having the highest inhibitory zone diameter for B. subtilis (25 mm), S. aureus (21 mm), and C. albicans (14 mm) were disagree with the current study.

In addition to the environmental factors, the presences of different secondary metabolites have contributed to varied antimicrobial properties. Our finding confirmed that all the study plants contain secondary metabolites such as flavonoids and majority of the study plants contain alkaloids, glycosides, and tannins. Hence, presence of these metabolites considerably important for antifungal activities through

different mechanisms. The study conducted by Shimada (2006) reported that tannins have been found to form irreversible complexes with proline rich protein resulting in the inhibition of microbial protein synthesis. Others like alkaloids, saponins, flavonoids and glycosides were found to have in-vitro antimicrobial properties (Fridous et al. 1990). Therefore, in our finding presence of these different phytochemicals supports the practice of traditional healers for treatments of *Malassezia furfur* in human and formation of decay in fruit and vegetable. Other study indicates that the activities observed could be due to the presence of some of the secondary metabolites like, alkaloids, tannins, sterols, glycosides, saponins, terpenes and flavonoids present in the plant (Egharevba et al. 2010).

5.3. Minimum inhibitory concentration of crude extracts

Minimum inhibitory concentration (MIC) is the lowest concentration of plant extracts at which the extracts inhibit or kill the test fungal species. In the current study five crude extracts were tested for MIC values. The lowest MIC values for all the crude extract of selected medicinal plants were 3.5 mg/ml while the highest was 100 mg/ml. This indicates that the plant extract exhibited antifungal activities. In some plant extracts and fungal species the current result indicated that the resistance of the fungal species and low potency of some of the crude plant extracts. The fact that there was decreased antimicrobial activity with decrease in concentration of the extract, suggests that the antimicrobial effect of the three medicinal plant extracts is concentration-dependent. The 100 mg/ml concentration of the extract gave the highest inhibition zone for the three inhibited fungal species which suggests that plant extracts exhibits the best antimicrobial effect at this (100 mg/ ml) concentration. The inhibition zone for each organism at 70-3.5 mg/ml concentration is lower when compared with those of 100 mg/ml concentration. This result may suggest that the more the concentration of phytochemicals responsible for the antimicrobial activity, the better the effect. Another study on the activities of medicinal plant on microorganism indicates that minimum inhibitory concentration ranging from 1.25 – 5 mg/ml (Egharevba et al. 2010) which were disagree with the present study. On the other hand the study conducted by Udegbunam et al. (2015) indicated that the minimum inhibitory concentration ranges from 100 mg/ml-12.5 mg/ml it has been near to the current study.

5.4. Preliminary phytochemical screening of selected medicinal plants

In the present study, all the three selected medicinal plants were evaluated for their phytochemical constituents. That means the availability of alkaloids, glycosides, flavonoids, saponins, and tannins were tested. None of the studied medicinal plants constitute all of the described secondary metabolites. Only Flavonds was found in the three medicinal plants and *Laggera tomentosa* was tested positive for four type phytochemicals such as alkaloids, tannins, flavonds and glycosides. The antimicrobial activity of medicinal plants could be attributed to its alkaloids, glycosides, flavonoids, saponins, phenol and tannins content as revealed by different authors done on phytochemical analysis (Udegbunam et al. 2014; Udegbunam et al. 2013; Goncalves et al. 2009). The activities observed could be due to the presence of some of the secondary metabolites like, alkaloids, tannins, sterols, glycosides, saponins, terpenes and flavonoids present in the plant (Egharevba et al. 2010) and other phytochemicals such as a phenolic alkaloid contained in medicinal plants have been reported to exhibit antimicrobial activity (Adesanya et al. 1992). These idea means that the antimicrobial activity of plant extracts depends on the type and amount of phytochemicals present in the plant tissue and pathogens inherent resistance (Martin et al. 2004). Phytochemicals are the wide variety of compounds produced by plants and manipulated wisely in the pharmacognostic drug development and used for treatment of the major ailments (Sivasankari et al. 2010).

In the current study, highest zone of fungal inhibition was observed extracts derived from *Datura stramonium*, the type and the inclusiveness of phytochemicals could have a contribution to its effective activity against the test species compared with other plants (Ali & Blunden, 2003).The possession of varieties of microbial active phytochemical accounts for the efficacy of this traditional medicinal plant, hence preference of traditional healers to the plant although done without scientific ground of its activity. Plant extracts containing tannins have been widely reported to inhibit the growth of fungal species (Vasconcelos et al. 2003; Vasconcelos et al. 2006; Anibal et al. 2013). Failure to inhibit the growth of microorganisms could be attributed to its inherent resistance of the microorganism to antimicrobial agents (Li et al. 1994). Reports on the phytochemical constituents are not consistent. For instance, Eyong et al. (2011), evaluated Vernonia amygdalina phytochemicals and obtained positive result for Alkaloids, Tannins, Flavonoids, and Saponins. To the contrary Ogundare (2011) reported the absence of alkaloid in the leave of

Vernonia amygdalina. The differences in the reports of many of the study could be due to the difference in time of plant collection, climate, methods of extraction used and other factors.

6. Conclusion and recommendations

6.1. Conclusion

Based on the results found in this study, all of the medicinal plants have shown different activities against the test species. The activities observed could be due to the presence of some of the secondary metabolites like, alkaloids, tannins, glycosides, saponins, and flavonoids present in the plant and other phytochemicals such as a phenolic alkaloid contained in medicinal plants. These idea means that the antimicrobial activity of plant extracts depends on the type and amount of phytochemicals present in the plant tissue and pathogens inherent resistance. However, the degree of antifungal activity influenced by the suitable solvents used for extraction; it could also depend up on the condition or state of the sample along with the season in which the plant was collected. Several factors such as age of the plant, duration of storage, temperature, pH, preparation of media could also directly or indirectly affect the activities of extracts on the fungal species. On the other hand the antifungal activities of the crude extracts of all the selected medicinal plants were dependent of concentrations. At higher concentration best activities were observed for many extracts however when the concentration decreased the antibacterial activities also decrease. There may be several factors which can affect or reduce the antimicrobial activities of medicinal plants such as types of collection of the plants, storage and extraction process.

6.2. Recommendations

Based on the results of this study the following recommendations are suggested:

The present results indicates that *Datura stramonium* chlorofrm extract (DSC) and *Datura stramonium* acetone extract (DSA) have highest activity against *Penicillium expansum* with zone of inhibition 15.6±1.5 and 14±0 respectively. Hence, further high performance liquid chromatography (HPLC and mass spectroscopy (MS) analysis should be better in order to identify to which active compound related to fungicidal power and to know the structure of respective compound. The current work was done using only three solvents such as petroleum ether, chloroform, and acetone. Thus, to make the generalization on the activity spectrum of the plants extract more evidential, it could be evaluated by other more solvents for extraction of the active compounds. In addition to these it could be better to check other phytochemicals for the antifungal activities.

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