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VARIATIONS IN PHENOLICS, ANTIOXIDANT AND ANTIFUNGAL ACTIVITIES AMONG DIFFERENT PARTS OF SELECTED MEDICINAL PLANTS

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Abstract

The extracts, produced by 80% methanol, from leaf, bark and seed of three medicinal plants namely neem (*Azadirachta indica* A. Juss), kiker (*Acacia nilotica* L.) and jaman (*Eugenia jambolana* L.), were assessed for their total phenolics (TP), total flavonoids (TF) and antioxidant and antifungal activities. Appreciable quantities of TP and TF, ranging from 24.43-176.16 mg GAE/g DW and 16.33 to 41.92 mg CE/g DW, respectively, were established in different parts of the selected plants. Antiradical potential evaluated in terms of DPPH radical scavenging activity ranged from 34.02 to 71.54%, inhibition of linoleic acid peroxidation, 60.16 to 76.53% while reducing power (2.5 to 10 mg/mL concentration) 0.55 to1.49. Antifungal activity of the extracts was examined against two fungal strains *viz. Aspergillus flavus* and *Aspergillus parasiticus* using disc diffusion method and micro dilution broth susceptible assay. Among the three medicinal plants selected, the crude extract from neem leaves was found to be the most potent against the tested fungal strains as well as exhibited greater antioxidant activity ($p<0.05$).

Introduction

Lipid oxidation not only causes different health disorders but also leads to development of bad odors, changes in the taste and texture as well as losses of nutritional and organoleptic value of foods (Babbar *et al*., 2011). On the other hand, decay and spoilage of foods by some pathogenic and toxigenic fungi during storage is another serious problem, especially in the humid, hot and tropical regions of the world. Such environmental conditions favor the colonization of various fungal species in agriculture crops and stored foods especially in cereals and grains leading to mycotoxins contamination. *Aspergilli* are the most common and toxigenic fungi as they can produce toxic chemical aflatoxins in food and feed stuff. Among more than 300 mycotoxins, produced by different molds, aflatoxins are the most toxic carcinogenic for mammals and human beings (Beltran *et al*., 2011; Mushtaq *et al.,* 2012).

Many crops including cereals, grains, fruits and vegetables, due to containing moisture, protein and fiber, are highly susceptible to mycotoxin contamination and as result their nutritive quality and organoleptic value is decreased. According to FAO estimate, world over 25% crop production with estimated cost of US$100 million and in European Union (EU) 20% of crop production may be contaminated by fungi and their toxic metabolites that are linked to developing serious disorders to human and animals health (Murphy *et al*., 2006). Aflatoxins control through chemical treatment is an effective and efficient strategy but restrictions are being executed by food and regulatory agencies on the chemicals use due to their perceived toxicity and carcinogenicity. Meanwhile, another important concern is that several aflatoxins are gaining resistance against synthetic compounds due to their long term uses. Therefore, to meet such challenges, the researchers are seeking some methods involving the use of plant based natural, safer, and cost-effective alternatives to protect foods from microbial contamination (Haciseferogullary *et al*., 2005).

Currently, the applications of phytochemicals as antioxidant and antimicrobial agents for food preservation, is gaining much importance due to their potential health and nutraceutical benefits in contrast to synthetic chemicals which may pose some health-related threats (Thanaboripat *et al*., 2000; Hamza *et al*., 2006; Hayouni *et al*., 2007; Mehmood *et al.,* 2013). Many studies have shown that medicinal and aromatic plants are rich source of phytochemicals with multiple biological effects such as antioxidant, anti-inflammatory, microbicidal and pesticidal (Bobbarala *et al*., 2009; Ghimeray *et al*., 2009; Hussain *et al.,* 2011). Due to multiple biological functionalities and health benefits, especially, natural antioxidants such as polyphenols are gaining much importance as ingredients for functional food and nutraceuticals (Hayouni *et al*., 2007). On the basis of modern scientific research, several plant extracts and essential oils have been found to act as good antifungal agents against toxigenic fungi and thus can suppress the synthesis of toxic chemicals in foods. For example, *Azadiracha indica* extract has been reported to have good inhibitory effect on fungal growth and mycotoxin production (Reddy *et al*., 2009).

Nature has ornamented and blessed Pakistan with huge reserves of medicinally and/or economically important flora and fauna. There are thousands of potential plants which could be explored for bio-prospecting and value-addition through their utilization for the development of functional foods and natural therapeutic drugs/agents. Different parts of the local neem, kiker and jaman plants have not yet been quantified for phenolic components and biological attributes. This urged us to quantify amounts of total phenolics and total flavonoids and assess antioxidant and antifungal properties of different parts of selected medicinal plants commonly used in the traditional medicine system of Pakistan.

Materials and Methods

Sample collection: The samples of leaves, seeds and bark were harvested from the mature plants of neem (*Azadiricha indica* A. Juss), kiker (*Acacia nilotica* L.) and jaman (*Eugenia jambolana* L.) grown in the locality of Faisalabad, Pakistan. The samples were hot-air dried, pulverized into fine powder and preserved in air-tight polythene zippers.

Fungal strains: The strains of mold i.e., *Aspegillus flavus* and *Aspergillus parasiticus* were procured from Fungal Culture Bank of the Department of Plant Pathology, UAF, Faisalabad, Pakistan. The strains were cultivated (at 28^{o}C) PDA (potato dextrose agar) (Oxoid, UK) and then isolated and purified for further antifungal tests.

Extraction of antioxidant/antifungal components: The hot air-dried plant samples were grounded (80 mesh) to fine powder using a grinder. Twenty grams of each of the ground plant material was extracted overnight with 200 mL of aqueous methanol (80%) under ambient conditions using an orbital shaker (Gallenkamp, UK). The resultant mixture was passed through Whattman filter paper No. 1 to separate filtrates while the sediment left behind was re-extracted twice via the same procedure. The two extractions were combined and subjected to excess-solvent removal under reduced pressure (45^{o}C) with the help of a rotary evaporator (Eyela, Tokyo, Japan). The calculation of yield of extractable components was based upon the weighed amount of solvent-free crude extract. The dried extracts were quantitatively transferred into brown sample vials and preserved at 4^{o}C until used for further experiments.

Determination of total phenolics and total flavonoids: TP contents were estimated colorimetrically using Folin-Ciocalteu (FC) as a reagent (Chaovanalikit & Wrolstad, 2004). In this assay, 5 mg of the subject extract, placed in a test tube, was diluted with 7.5 mL deionized water and mixed with 0.5 mL of FC reagent. After 10 min incubation at room temperature, 5 mL of 20% sodium carbonate was added and the contents of the test tube were heated at 40^{o}C and then chilled using ice bath. Finally, absorbance of the reaction mixture (solution) was recorded at 755 nm. The phenolic contents were estimated using Gallic acid calibration curve and the data generated were expressed as Gallic acid equivalents (mg GAE/g dry weight).

Total flavonoid contents (TFC) were measured using slightly modified method as revealed in a report by Dewanto *et al*., (2002). Briefly, extract of each plant (1.0 mL with concentration 0.1mg/mL) was taken in a volumetric flask and diluted with 4 mL of distilled water. Initially, 0.3 mL of 5% $NaNO_2$, 0.3 mL of 10% $AlCl_3$ and 2mL of 1.0 *M* NaOH were added at 0, 5 and 6 min interval, respectively. The contents were then diluted with 2.4 mL distilled water, mixed vigorously, followed by absorbance reading at 510 nm with a spectrophotometer. TFC were expressed as catechin equivalents (mg CE/g dry weight).

DPPH free radical scavenging activity assay: The free radical [(2, 2'-diphenyl-1-picrylhydrazyl (DPPH)] scavenging activity of methanolic extracts of different parts of selected plant materials was estimated based upon an earlier procedure (Iqbal *et al*., 2005). In this assay, 5.0 mL of freshly prepared DPPH solution (0.025 g/L) was added to 1.0 mL of crude extract (25 µg/mL of dry weight in methanol). Absorbance at 515 nm was measured at different time intervals (0, 0.5, 1, 2, 5 and 10 min) colorimetrically. A comparison among the absorbance data was made at different time intervals and by using the absorbance measured at 5th minute that corresponded to maximum change; the values of radical scavenging in percentage were calculated.

Inhibition of linoleic acid peroxidation and reducing power: The antioxidant efficacy of the tested plant extracts in terms of their potential to inhibit the oxidation of linoleic acid was also assessed (Iqbal *et al*., 2005). Briefly, five grams of each plant extract was separately added to 0.13 mL of linoleic acid (0.13 mL), 10 mL of 99.8% ethanol, 10 mL of 0.2 *M* sodium phosphate buffer (pH 7). Now, 25 mL of distilled water was added to the mixture followed by incubation for 360 hours in an oven at temperature of 40^{o}C. The extent (degree) of oxidation was measured following isothiocyanate method as described by Yen *et al*., (2000) while the inhibition of oxidation was calculated in percentage.

The reduction potential of plant extracts to reduce Fe^{+3} into Fe^{+2} was assessed following the modified procedure as described by Yen *et al*., (2000). Briefly, extract solutions having 2.5, 5.0, 7.5 and 10.0 mg contents of extract dissolved per mL of 70% aqueous ethanol were mixed with 5.0 mL of 1.0% potassium ferricyanide at pH 6.6 (5 mL of 0.2 *M* sodium phosphate buffer). The resulting mixture was kept at 50^{o}C for 20 min, mixed with 5 mL of 10% trichloroacetic acid, centrifuged for 10 min (980 *g*) at 5°C in a refrigerated centrifuge machine leading to separating the layers. The upper layer separated was diluted by adding distilled water and 1.0 mL ferric chloride, (0.1%) and the absorbance of this final reaction mixture was recorded at 700 nm using a spectrophotometer to estimate the reducing potential.

Antifungal activity of plant extracts

Disc diffusion method and microdilution broth method: The plant extracts were tested against two aflatoxin producing strains namely *Aspegillus flavus* and *Aspergillus parasiticus* by disc diffusion method (Anon., 2007). The autoclaved potatoes dextrose ager (PDA) media was inoculated with the test fungi and poured in petri plates to allow for solidification. Six mm diameter sterilized paper discs saturated with 50 µL of plant extract were placed on the surface of PDA and extract compounds were allowed to diffuse for 5 min and plates were incubated at 28^{o}C for 48 h. In the case of fungal strains Terbinafine HCl was used as positive and disc having no extract sample as the negative control, respectively. The inhibition zone around the disc was calculated by zone reader in millimeter to evaluate the antifungal activity.

The concentration, required to completely inhibit the growth of microorganism *i.e*. minimum inhibitory concentration (MIC), was determined with the help of microdilution broth susceptibility assay (Anon., 2007). Microtiter plate reader (Biotech USA) was used to determine the growth rate of fungi by measuring the optical density at 620 nm (OD 620) as described by Kaiserer *et al*., (2003). A series of dilutions were prepared in the concentration range of 10-100 µg/mL of extract in micro titer plate, including one growth control. SDB (160 uL) was added on to the micro plate with 20µL of test solution. Then 20 µL of 5×10^5 CFU/mL of the *A. flavus* and *A. parasiticus* fungi suspension was inoculated on to the separate micro plate. The plates were incubated at 28°C for 24 h and then shifted at 22°C to avoid rapid overgrowth of the untreated controls. The optical density was determined at 620 nm using a micro titer plate reader and then MIC (µg/mL) was calculated from the optical density versus concentration curve constructed.

Statistical analysis: All the experiments/measurements were carried out in thrice and the results of the tested parameters were given as mean of triplicate experiments ± SD. Data obtained was analyzed for variation among the plants and fungal strains investigated using Statistica 8.1 (Stat Soft Inc., Tulsa Okahoma, USA). While $p<0.05$ was used to consider the means to be statically significant.

Results and Discussion

Extract yields: The extraction yields of 80% methanol (methanol:water, 80:20 v/v) soluble components (MSC) from different parts of the selected medicinal plants are presented in Table 1. The extract yields from leaves, bark, and seeds of different medicinal plants varied over 14.61-40.32% indicating a significant (p<0.05) variation among plants tested. The maximum yield (40.32%) was obtained from neem seeds while the minimum (14.61%) in neem bark. Among different parts of medicinal plants, the highest yields were recorded for seeds (18.77-40.32%) followed by leaves (15.66-29.04%) and then bark (14.61-30.96%). Variation in the yields of extractable components among different plant parts may be linked to varying chemical nature of the compounds present in leaves, bark and seed (Jayaprakasha *et al*., 2001).

The yields of extracted components, apart from their chemical composition, are also affected by the polarity, solubility and the concentration and nature of the extraction solvent. Hence, a proper solvent system has to be used for extraction/recovery of maximum amount of potent antioxidant components from a typical plant material. Pure methanol and ethanol as well as the aqueous mixture of these alcohols are widely recommended to extract and isolate plant antioxidants due to their compatible polarity and solubility for such natural compounds. According to Shon *et al*., (2004) aqueous methanol was noted to be efficient to extract antioxidant components from *Phellinus baumii*.

Total phenolic and total flavonoid contents: The amounts of total phenolics and total flavonoids, in methanolic extracts form different parts (leaves, seed and bark) of the selected medicinal plants ranged from 24.43 to 176.16 mg GAE/g DW and 16.33 to 41.92 mg CE/g DW, respectively (Table 1). Significant (p<0.05) variation was observed in total phenolic contents among the different plant parts tested. Neem leaves exhibited maximum amount of TP (176.16 mg GAE/g DW) while minimum for jaman seed (24.43 mg GAE/g DW). The higher values of TPC in neem leaves (176.16 mg GAE/g DW) and kiker leaves (175.54 mg GAE/g DW), as investigated in the present study, were in close agreement to those reported in pomegranate peel extract (161.25 mg GAE/g DW), (Kanatt *et al*., 2010) citrus unshiu var. Ishikawa peels (195.5 mg GAE/g DW), *Citrus sinesus* var. Washington Navel peel (160.3 GAE mg/g) (Ghasemi *et al*., 2009). Meanwhile, TPC of neem bark (106.70 mg GAE/g DW), and neem seed (104.56 mg GAE/g DW) were found to be higher than those reported for mango (54.67-109.70 mg GAE/g) (Ajila *et al*., 2010) and jaman bark (78.00-128 mg GAE/g) (Sultana *et al*., 2007a). The level of total phenolics in different parts of medicinal plants reported in this study was found to be lower than that investigated in leaf and bark of neem 126.72 mg/g and 651.07 mg/g, respectively (Ghimeray *et al*., 2009).

Table 1. Extract yield, total phenolic (TP) and total flavonoid (TF) contents in different parts of selected medicinal plants.

Plant part	Extract yield (g/100g)	TP (mg GAE/g DW)	TF (mg CE/g DW)
Neem leaves	18.55 ± 0.35	176.16 ± 3.54	38.60 ± 0.81
Neem seeds	33.02 ± 0.63	104.56 ± 2.32	26.91 ± 0.64
Neem bark	14.61 ± 0.25	106.70 ± 2.21	35.61 ± 0.71
Kiker seeds	40.32 ± 0.79	99.10 ± 1.99	31.64 ± 0.63
Kikar bark	30.96 ± 0.62	124.65 ± 3.18	32.60 ± 0.69
Kikar leaves	29.04 ± 0.60	175.54 ± 3.23	41.92 ± 0.82
Jaman seed	18.77 ± 0.40	24.43 ± 0.49	16.33 ± 0.39
Jaman bark	18.90 ± 0.39	91.99 ± 1.68	21.61 ± 0.45
Jaman leaves	15.66 ± 0.33	94.02 ± 1.79	29.25 ± 0.63

Values are mean ± SD of three separate experiments

As far as the concentration of total flavonoids is concerned, the highest contents of these antioxidant compounds were determined in kiker leaves (41.92 mg CE /g), while the lowest in jaman seed (16.33 mg CE /g). These amounts of flavonoids were comparable to those reported for bark and leaf extract of neem (14.21mg/g and 32.50 mg/g) (Ghimeray *et al*., 2009), pomegranate peel extract (7.57 mg CE /g) (Kanatt *et al*., 2010), and limonella apple peel (47.8 mg/100g QE) (Abrosca *et al*., 2007), but were higher than those reported by Ghasemzadeh *et al*., (2010) for methanolic extracts of different parts of two varieties of young ginger (1.30-7.05 mg quercetin/g DW). The present trends for phenolics in different parts of medicinal plants were in agreement to the findings of Karimi *et al*., (2011) who also reported greater amount of TP and TF in the leaves than the stem. A larger amount of phytochemicals (TPC and TFC) in leaves might be the result of photosynthesis which mainly took place in this part of plants (Silva *et al*., 2006). Flavonoids, considered as the plant secondary metabolites with multiple biochemical and antioxidant properties, are naturally distributed in many fruits, flowers, vegetables, grapes and grapes byproducts and coffee etc., (Benbrook 2005; Sultana *et al*., 2008). They provide protection against carcinogenesis by suppressing the free radical formation and oxidative stress related disorders. Flavonoids have been recognized as strongest natural antioxidants on the basis of their ability to scavenge free radicals and reactive oxygen species (Ghasemzadeh *et al*., 2010).

Antioxidant activity: Due to the complexity of antioxidants mechanisms, antioxidant activity of plant extracts is usually evaluated by using more than one test. In the present work, therefore, different antioxidant assays were used to probe the antioxidant potential of the tested medicinal plant extracts.

DPPH radical scavenging activity: DPPH, a stable radical, has been widely used to evaluate the free radical scavenging activity of botanical extracts (Ozturk *et al*., 2007). DPPH free radical assay is not only used to asses electron or hydrogen atom donating properties of antioxidants and phenolic compounds but also evaluates the rate of their reaction towards the free radicals. DPPH radical, having deep violet color, shows absorption maxima at 515-528 nm, and when it receives proton from any hydrogen donor species such as phenolics, it loses its chromophoric nature and converts into yellow color and this change is directly linked with concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds. As DPPH radical scavenging activity increases, antioxidant activity also increases.

DPPH scavenging activity for different extracts of selected medicinal plants ranged from 34.02-71.54% (Table 2). The results showed that among the different parts of the investigated plants, neem leaf extract possessed highest activity to scavenge DPPH (71.54%) followed by kiker leaf and jaman leaf with contribution at 66.54% and 54.27%, respectively. The variation in scavenging power among different medicinal plant parts could be attributed to the presence of varying amount of bioactive compounds such as phenolics, flavonoids and tannins (Ghimire *et al*., 2011). The present results showed that mostly the extracts with high content of phenolics and flavonoids exhibited greater power to scavenge free radicals but some extracts despite of less amount of phenolics depicted appreciable activity suggesting that the presence of some other secondary metabolites (carotenoids, volatile oils and vitamins) may also contribute towards scavenging capacity (Yingming *et al*., 2004; Odabasoglu *et al*., 2005). Previously, Ghasemzadeh *et al*., (2010) observed a strong correlation of radical scavenging power of plant extract with TP and TF.

The present DPPH scavenging capacity, ranging from 34.02-71.54%, was in close agreement with those investigated for barks of some trees namely neem, kiker, arjun and jaman (49.0%-87.0%) (Sultana *et al*., 2007a) and apple pulp (69.01%) (Leontowicz *et al*., 2003). The highest scavenging activity (71.54%) of neem leaf was in close agreement with the findings of Ghimire *et al*., (2011) who reported 73.67% scavenging capacity for *Azadirachta indica* (neem) leaf.

Table 2. Antioxidant activity of extracts from different parts of selected medicinal plants.

Plant part	DPPH radical scavenging activity (%)	Inhibition of linoleic acid oxidation (%)
Neem leaves	71.54 ± 1.49	76.53 ± 1.59
Neem seeds	40.42 ± 0.89	63.32 ± 1.22
Neem bark	43.62 ± 0.91	70.45 ± 1.61
Kiker seeds	38.65 ± 0.74	67.23 ± 1.17
Kikar bark	42.42 ± 0.89	72.34 ± 1.51
Kikar leaves	66.45 ± 1.34	76.37 ± 1.46
Jaman seed	48.01 ± 1.25	60.16 ± 1.21
Jaman bark	34.02 ± 1.15	65.04 ± 1.23
Jaman leaves	54.27 ± 1.27	70.48 ± 1.43

Values are mean ± SD of three separate experiments

Inhibition of linoleic acid peroxidation: In this assay, antioxidant activity (AA) of the extracts was followed by assessing their potential towards inhibition of peroxidation in linoleic acid system (Yen *et al*., 2000). Higher absorbance in this assay correlates with higher concentration of peroxides formed during the reaction, consequently lower the antioxidant activity. A considerably high magnitude of inhibition of peroxidation, ranging between 60.16% and 76.53%, was noticed for extracts from different parts of medicinal plants (Table 2) that might have been attributed to the presence of antioxidants, such as phenolics and flavonoids that are responsible for the antioxidant activity of the botanical (herbal) extracts (Yen *et al*., 2000). Among the plants analyzed, the highest level of inhibition of linoleic acid peroxidation, 76.53% and 76.37%, was exhibited by neem and kiker leaves extracts. Jaman leaves although have comparatively low total phenolic contents but exhibited high inhibition of linoleic acid peroxidation indicating that some bioactives such as tannins, terpenoids, steroids, and ascorbic acid etc., other than the phenolics might have contributed to this activity (Gowri & Vasantha 2010). This inhibition potential of the tested plant extracts was found to within the range of extracts from corncob (37.3-89.9%), barks of neem, kiker, arjun, and jaman (44.04% to 90.02%) (Sultana *et al*., 2007a; Sultana *et al*., 2007b). The efficacy of medicinal plant extracts to inhibit oxidation of linoleic acid reflects a complex composition (nature) of plant materials (hydrophilic versus hydrophobic nature of compounds) that enables these to interact with emulsion components (Ghimeray *et al*., 2009).

Reducing power: Measurement of reducing potential can also be used to assess some antioxidant properties of plant extracts. As result of reduction by the antioxidant compounds of extract the yellowish ferric cyanide solution was transformed into bluish green ferrous cyanide complex and the intensity of color was noted spectrophotometerically at 700 nm. The intensity of color is supposed to be directly related to the reducing power of extract, and ultimately reflects antioxidant behavior (potency) of the plant material investigated (Zuo *et al*., 2004). The reducing power of bioactive compounds is related to their ability to transfer electron resulting into reduction. The reducing potential of methanolic extracts of different parts of selected medicinal plants is shown in Fig. 1. The absorbance of the tested plant extracts, recorded over a range of 0 to 10 mg/mL, revealed the reducing potential to be directly concentration dependent. The reducing potential (absorbance values) of the tested plant extracts at 10 mg/mL varied from 0.55-1.49. The highest reducing power (1.49) was observed for kiker leaves extract whereas lowest (0.55) for jaman seed extract.

The values of reducing power as determined in the present analysis for kiker leaves were found to be comparable to those reported for methanolic bark extracts of *Azadirachta indica* (1.46), *Terminalia arjuna* (1.60), *Acacia nilotica* (1.52) and *Eugenia jambolana* (1.48) (Sultana *et al*., 2007a). The present reducing data (0.55-1.49 for concentration 10 mg/mL)) were some what comparable with the findings of Babbar *et al*., (2011) and Ribeiro *et al*., (2008), who investigated the reducing power of extracts from six fruits residues and four mango varieties to be 0.31 to 1.54 and 0.42-1.27, respectively. The present variation in antioxidant activity among different medicinal plant parts tested may be attributed to the varying amounts of phenolics which mainly act as reducing agents (Ghimeray *et al*., 2009).

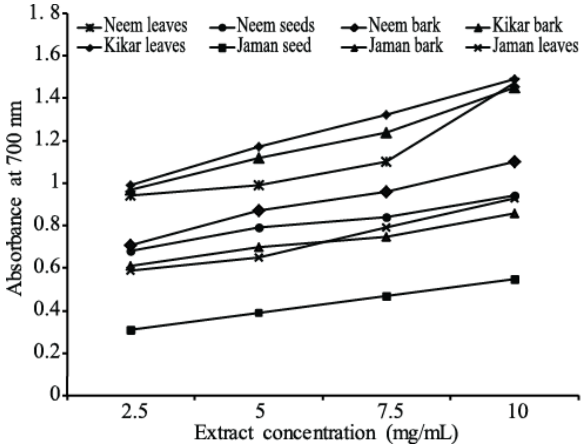

Fig. 1. Reducing potential of extracts from different parts of selected medicinal plants.

Antifungal activity: The results of antifungal activity of methanolic extracts from different parts of selected plants, against two pathogenic fungal strains *Aspergillus flavus* (*A.flavus*) and *Aspergillus parasiticus* (*A.parasiticus*), recorded by disc diffusion method, are shown in Table 3. In general, all the extracts exhibited inhibitory effect against *A.flavus* and *A.parasiticus* with diameter of inhibition zone (DIZ) between 17-29 mm and 15-32 mm, respectively. There was significant difference for the efficacy of plant extracts against both the strains of fungi. Among the plants studied, neem extracts and kiker extracts showed higher antifungal activity against both the tested fungi than that of jaman extracts. The control (without extracts) did not inhibit the growth of any of the fungal strain.

Out of the nine plant materials tested, the extracts from neem leaves, kiker leaves, and kiker bark greatly inhibited the growth of both the tested fungi with DIZ values 29, 26, and 25, mm for *A. flavus* and 32, 25 and 26 mm for *A. parasiticus,* respectively. Whereas a moderate inhibitory effect was observed by neem seed, neem bark, and kiker bark extracts against *A. flavus* and *A. parasiticus* with DIZ 21, 24, and 25 and 22, 23, and 26, mm, respectively. Such variations in antifungal activity among plants parts might be linked to the varying concentrations of antifungal agents present in the plant parts analyzed. According to reports, antimicrobial potential of plants is basically defined by the chemical composition and nature of specific material/specific parts, e.g., saponins are present in ginseng while roots contain only essential oil on the other hand, in Eucalyptus essential oil is mainly present in leaves. Whereas in balsamic poplar active phytochemicals are confined in sprouts, leaves, and stems (Sanchez *et al*., 2005).

The inhibitory effect of selected plant extracts against *A. flavus* (17-29 mm) was comparable to that observed against *A. parasiticus* (15-32 mm). The highest antifungal effect of neem leaves, kiker leaves and kiker bark, against *A. flavus (*29, 26, and 25, mm) and *A. parasiticus* (32, 25 and 26 mm) was found to be significantly higher than those reported for *Holarrhena antidysenteria* bark (11mm and 16 mm) and *syzygium jambolamum* seeds (12 mm and 11mm) against *A. flavus* and *A. niger* (Parekh & Chanda, 2008; Chandrasekaran & Vekatesalu, 2004), whereas it was found to be comparable to those reported for alcoholic extract of neem leaves at concentration of 0.5% (42.10 mm) against *Aspergillus* and garlic bulb (42 mm) and Chinese leek (37.8 mm) against *A.niger* (Mondali *et al*., 2009; Yin & Tsao, 1999). The inhibitory effects recorded for the tested medicinal plant extracts (methanolic) against both *Aspergillius* species in the present study were found to be weaker than that of methanolic extract (at concentration of 100 mg/mL) of *Acacia nilotica* (kikar) (93.35 mm) against *A. flavus* but were in close agreement to those reported in scallion against *A.niger* (12.6 mm), *A.flavus* (14 mm), singara rind *A. flavus* (15 mm), pomegranate rind (23 mm) and bakeri garlic (22 mm) (Satish *et al*., 2007; Daham *et al*., 2010).

The crude extracts of neem and kiker showed good antifungal activity against both the tested fungi as revealed by minimum inhibitory concentration (MIC). The MICs were determined as the lowest concentration of extracts that completely inhibited the growth of fungal spores (Table 3). The tested plant extracts showed a wide range of MICs against both the tested fungi. The MICs for the tested extracts against *A. flavus* ranged from 381 µg/ mL to 835 µg/ mL while against *A. parasiticus* 181 µg/ mL to 965 µg/ mL. The lowest MIC values (181 µg/ mL) were observed for neem leave against *A.parasiticus* whereas the highest (965 µg/ mL) was observed for jaman seed extract against *A.parasiticus*.

Table 3. Antifungal activity (diameter of inhibition zone in mm) and minimal inhibitory concentration (MIC) of extracts from different parts of selected medicinal plants against two *Aspergillus* species.

Plant part	Diameter of inhibition zone (mm)		MIC (µg/mL)	
	Aspergillus flavus	*Aspergillus parasiticus*	*Aspergillus flavus*	*Aspergillus parasiticus*
Neem leaves	29.00 ± 0.63	32.00 ± 0.61	381 ± 7	181 ± 4
Neem seeds	21.00 ± 0.39	22.00 ± 0.43	520 ± 11	595 ± 11
Neem bark	24.00 ± 0.42	23.00 ± 0.47	452 ± 8	490 ± 9
Kiker seeds	22.00 ± 0.30	21.00 ± 0.40	517 ± 11	550 ± 11
Kikar bark	25.00 ± 0.28	26.00 ± 0.55	407 ± 8	371 ± 7
Kikar leaves	26.00 ± 0.56	25.00 ± 0.49	395 ± 7	399 ± 6
Jaman seed	20.00 ± 0.45	15.00 ± 0.25	465 ± 9	965 ± 19
Jaman bark	19.00 ± 0.42	18.00 ± 0.39	665 ± 13	802 ± 16
Jaman leaves	17.00 ± 0.38	19.00 ± 0.41	835 ± 17	626 ±13

Values are mean ± SD of three separate experiments

There is no authentic criterion for MIC end points *In vitro* testing of plant extracts but according to Aligiannis *et al*., (2001) on the basis of MIC values plant can be classified as: strong inhibitors with MIC up to 500 µg/mL; moderate inhibitors having MIC between 600 to 1500 µg/ mL and weak inhibitors with MIC greater than 1600µg/ mL. Based on above criteria neem leave, kiker leave, neem bark, kiker bark, showed strong activity against both the tested fungi. The MIC values of neem leaves and kiker laves extracts against both the tested fungi *A. parasiticus* and *A. flavus* in the present study (181 µg/ mL, 381 µg/ mL and 395 µg/ mL, 399 µg/ mL, respectively), were comparable with MIC for aqueous extracts of *Syzygium jambolanum* seeds (250 µg/ mL) against *A. flavus,* and (250 µg/ mL) against *A. niger* (Chandrasekaran & Vekatesalu, 2004) but found to be lower than that investigated by Hamza *et al*., (2006) for *Acacia nilotica* (kiker) 1000 µg/ mL and 4000 µg/ mL against two fungi *Candida krusei* and *Cryptococcus neoformans,* respectively.

The present trends of inhibition showed that the tested plant extracts have different inhibitory effect against the growth of both the *Aspergillus* species suggesting the presence of different amounts of active antifungal compounds. This may be linked to varying nature of extractable components and further defined by the mechanisms of action against typical *Aspergillus* species. It has been reported earlier that tannins and phenolic compounds derived from plant are responsible for appreciable antioxidant and antimicrobial activity against bacteria and fungi (Banso & Adeyemo, 2007; Ozcan & Juhaimi, 2010).

Conclusions

According to the results of this study the extracts from the selected three medicinal plants showed considerable antioxidant and antifungal activity against selected aflatoxigenic and pathegenic fungal strains. Among the tested plants, neem leaf extract showed the highest antioxidant and antifungal activity followed by kiker leaf extract. The use of these medicinal plants would be helpful to control oxidative deterioration and contamination of foods and grains against *A. flavus* and *A. parasiticus* during storage and processing. Overall, the leave and bark extracts of the medicinal plants studied, having higher concentration of phenolics, were found to be more potent than the seeds extracts and thus can be explored as a valuable source of antioxidant and antifungal agents for the functional food and nutraceutical industry. However, further research is recommended to analyze and identify detailed profile of active substances of these plant materials using by HPLC/GCMS to ascertain their uses for specific food or pharmaceutical applications.

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