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, Azadirachta 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 bioprospecting 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°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°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°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°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).

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

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 (14.21 mg/g and 32.50 mg/g) (Ghimere 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., 2003). DPPH free radical assay is not only used to assess 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.

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

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 spectrophotometrically 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.](attachment:image)

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 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 (11 mm and 16 mm) and *syzygium jambolanum* seeds (12 mm and 11 mm) 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 *A. niger* (Mondali et al., 2009; Yin & Tsao, 1999). The inhibitory effects recorded for both medicinal plant extracts (methanolic) against both *Aspergillus* 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>
<thead>
<tr>
<th>Plant part</th>
<th>Diameter of inhibition zone (mm)</th>
<th>MIC (μg/mL)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><em>Aspergillus flavus</em></td>
<td><em>Aspergillus parasiticus</em></td>
</tr>
<tr>
<td>Neem leaves</td>
<td>29.00 ± 0.63</td>
<td>32.00 ± 0.61</td>
</tr>
<tr>
<td>Neem seeds</td>
<td>21.00 ± 0.39</td>
<td>22.00 ± 0.43</td>
</tr>
<tr>
<td>Neem bark</td>
<td>24.00 ± 0.42</td>
<td>23.00 ± 0.47</td>
</tr>
<tr>
<td>Kiker seeds</td>
<td>22.00 ± 0.30</td>
<td>21.00 ± 0.40</td>
</tr>
<tr>
<td>Kiker bark</td>
<td>25.00 ± 0.28</td>
<td>26.00 ± 0.55</td>
</tr>
<tr>
<td>Kiker leaves</td>
<td>26.00 ± 0.56</td>
<td>25.00 ± 0.49</td>
</tr>
<tr>
<td>Jaman seed</td>
<td>20.00 ± 0.45</td>
<td>15.00 ± 0.25</td>
</tr>
<tr>
<td>Jaman bark</td>
<td>19.00 ± 0.42</td>
<td>18.00 ± 0.39</td>
</tr>
<tr>
<td>Jaman leaves</td>
<td>17.00 ± 0.38</td>
<td>19.00 ± 0.41</td>
</tr>
</tbody>
</table>

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 Ailgiamis 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 1600 μg/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 leaves 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 pathogenic 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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**References**


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