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ORIGINAL ARTICLE

Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species

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Abstract Plants are rich source of therapeutic compounds that have tremendous applications in pharmaceutical industry. To find new sources of antimicrobial and antioxidant agents, methanol/chloroform and aqueous extracts of 61 medicinal plants were evaluated systematically. Antimicrobial activity was assessed against six bacterial and five fungal strains, while natural antioxidants were studied using reducing power (RP), total antioxidant capacity (TAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Six plants exhibited broad spectrum antibacterial activity while two exerted significant antifungal activity. Total phenolic content (TPC) of the samples varied from 20.2 to 85.6 mg/g dry weight (DW) in M/C extracts and 5.5 to 62.1 mg/g DW in aq. extracts, expressed as gallic acid equivalents (GAE). Total flavonoid content (TFC) varied from 2.9 to 44.5 mg quercitin equivalent (QE)/g DW of sample for M/C extracts and 2.4 to 37.1 mg QE/g DW for aq. extracts. The results showed that antioxidant activities of plant species varied to a great extent not only among extracts (M/C and aq.) but also between the assays used for antioxidant evaluation. Significant linear correlation ($p < 0.01$) of TPC with antioxidant activities suggested their contribution to antioxidant activity. Using high performance liquid chromatog-

Abbreviations: M/C extracts, methanol/chloroform extracts; Aq., aqueous; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TPC, total phenolic content; TFC, total flavonoid content; RP, reducing power; TAC, total antioxidant capacity; GAE, gallic acid equivalents; QE, quercetin equivalents; HPLC–DAD, high performance liquid chromatography with diode detector

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raphy coupled with diode array detector (HPLC–DAD), gallic acid and rutin were detected in most of plant extracts with significant antioxidant activities. Study identifies plants with antimicrobial and antioxidant properties which could be used for isolation of desired therapeutic compounds and to develop infusions, nutriceuticals and pharmaceuticals.

1. Introduction

Infectious diseases are one of the major problems of the world and almost 57 million people die because of these diseases worldwide every year (Fauci et al., 2005). In last three decades, a number of new antibiotics have been produced by pharmacological industries but the toxic effects and the global emergence of multi-drug resistant (MDR) of microbes is limiting the effectiveness of these drugs (Hancock, 2005). On account of MDR efflux pump, there is a continuous need to sort out new and innovative therapeutic agents.

Reactive oxygen species (ROS) that are produced as a result of cellular metabolism are highly toxic and are involved in the etiology of many chronic diseases due to oxidative damage to lipids, nucleic acids and proteins. Although an internal system of antioxidant exists in our body but to get rid of excessive free radicals, exogenous antioxidants are recommended (Yanishlieva et al., 2006). Antioxidants can be natural and synthetic, but due to toxic and carcinogenic effects, synthetic antioxidants, such as butylhydroxyanisole and butylhydroxytoluene are being replaced with natural antioxidants (Botterweck et al., 2000).

Medicinal plants have been used to treat human diseases for thousands of years because they have vast and diverse assortment of organic compounds that can produce a definite physiological action on the human body. Most important of such compounds are alkaloids, tannins, flavonoids, terpenoids, saponins and phenolic compounds. Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity (Inayatullah et al., 2012). A number of such compounds have been isolated from plants which could be used for the development of new drugs to inhibit the growth of bacterial and fungal pathogens and to quench ROS with possibly novel mechanisms of action and low toxicity to the host cell (Ahmad and Aqil, 2007).

The main aim of the present research was to study phytochemical content and possible antimicrobial and antioxidant activities of 61 medicinal plants of Pakistan. The potential of antioxidant and antimicrobial activities of the extracts from such plants are of great interest in food and pharmaceutical industry.

2. Materials and methods

2.1. Plant collection

Samples of 61 medicinal plants were collected from different areas of Pakistan and identified by Professor Dr. Rizwana Aleem Qureshi, Department of Plant Sciences, Quaid-i-Azam University, Islamabad. Voucher specimens were submitted to the herbarium of Quaid-i-Azam University for future reference. The plants were selected on the basis of local use of these plants in folk medicine.

2.2. Extraction

Each plant material, mentioned in Table 1, was dried and ground (40 g). Extraction of half of the plant material was carried out using 80 mL mixture of methanol:chloroform (1:1; M/C) following maceration for 7 days. Other half (20 g) was boiled in distilled water (aq.) for 5 h for preparation of aqueous extracts. Filtrate obtained after filtering each plant extract with Whatman filter paper 1 and was centrifuged for 15 min (10,000 rpm) and concentrated in rotary evaporator (BUCHI Rotavapor R-200) at temperature below 50 °C. The residue obtained was stored at 4 °C for further use.

2.3. Phytochemical screening

2.3.1. Qualitative analysis of phytochemicals

Major constituents of both the M/C and aq. extracts were screened qualitatively as per standard procedures described by Jamil et al. (2012a). Major constituents analyzed were alkaloids, tannins, terpenoids, saponins, anthraquinones, cardiac glycosides and coumarins.

2.3.2. Quantitative analysis of phytochemicals

2.3.2.1. Total phenolic content. Total phenolic content of the extracts of 61 medicinal plants was determined spectrophotometrically according to Folin–Ciocalteu colorimetric method as reported by Haq et al. (2012). Sample concentration was 100 μg/ml and the absorbance was measured at 700 nm using spectrophotometer (Agilent, Germany). A calibration curve (0.0–25 μg/mL) was plotted using gallic acid and total phenolic content was expressed as gallic acid equivalents (GAE).

2.3.2.2. Total flavonoid content. Total flavonoid content of extracts (M/C and aq.) was determined using aluminum chloride colorimetric method (Chang et al., 2002). Sample (0.5 mL of 1.0 mg/mL methanol) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1.0 M potassium acetate and 2.8 mL of distilled water and was kept at room temperature for 30 min. Absorbance was measured at 405 nm. The calibration curve was drawn using quercetin as standard (0.0–8.0 μg/mL).

2.4. Antimicrobial activities

2.4.1. Antibacterial activity

Antibacterial activity of the extracts was determined against six bacterial strains, two gram positive i.e., *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538) and four gram negative i.e., *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 15224), *Bordetella bronchiseptica* (ATCC 4617) and *Enterobacter aerogens* (ATCC 13048) using the disc diffusion method as reported earlier (Jamil et al.,

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Table 1 Plants included in the present investigation and qualitative analysis of seven different classes of phytochemicals.

SN	Plant name	Local name	Family	Parts used	Place and time of collection	Terpenoids		Tannins		Coumarins		Quinones		Saponins		Alkaloids		Cardiac glycosides	
						[a]M/C	[b]Aq.	[a]M/C	[b]Aq.	[a]M/C	[b]Aq.	[a]M/C	[b]Aq.	[a]M/C	[b]Aq.	[a]M/C	[b]Aq.	[a]M/C	[b]Aq.
1	*Ageratum conyzoides* L.	Chick weed	Asteraceae	Leaves	Mianwali/March 2010	–	–	+	+	–	–	+	+	–	–	+	–	+	+
2	*Ajuga bracteosa* Wall.	Koribooti	Lamiaceae	Leaves	Quaid-i-Azam university/April 2010	+	–	+	+	+	+	–	–	–	+	+	+	+	+
3	*Albizia lebbek* L.	Frywood	Fabaceae	Bark	Islamabad/March 2010	–	–	–	–	–	–	–	–	+	+	+	–	–	–
4	*Anagallis arvensis* Linn.	Scarlet pimpernel	Myrsinaceae	Ariel parts	Mianwali/March 2010	–	–	+	+	+	+	+	+	+	+	+	+	–	–
5	*Anethum graveolens* L. *vr.White flower*	Dill	Apiaceae	Ariel parts	Mianwali/June 2010	+	+	+	+	+	+	–	–	–	–	+	–	+	–
6	*Anethum graveolens* L. *vr.yellow flower*	Dill	Apiaceae	Ariel parts	Mianwali/June 2010	+	–	+	–	+	–	–	–	+	+	–	–	–	–
7	*Artemisia roxburghiana* Wall. ex Besser	–	Asteraceae	Ariel parts	Nathiagali/May 2010	+	–	–	–	–	–	–	–	+	+	+	–	–	–
8	*Azadirachta indica* A. Juss.	Neem	Meliaceae	Leaves	Mianwali/May 2010	+	–	+	–	+	–	–	–	+	+	+	+	+	–
9	*Buxus papillosa* Schneider	–	Buxaceae	Leaves	Quaid-i-Azam university/April 2010	–	–	–	–	–	–	–	–	–	–	+	+	–	–
10	*Berberis lycium* Royle	Zarch	Berberidaceae	Bark	Nathiagali/April 2010	+	–	+	–	–	–	–	–	+	+	+	+	+	+
11	*Brassica campestris* L.	Mustard plant	Brassicaceae	Leaves, stem	Shahdara/March 2010	+	–	–	–	–	–	–	–	–	–	+	+	–	–
12	*Calendula arvensis* (Vaill.) L.	Field merigold	Asteraceae	Leaves	Samandwala/March 2010	+	–	+	–	–	–	–	–	+	–	+	–	–	–
13	*Calendula officinalis* L.	Zergul	Asteraceae	Leaves	Samandwala/March 2010	+	–	+	–	+	–	–	–	–	–	+	+	–	–
14	*Calotropis procera* (Aiton) W.T. Aiton	Ak	Apocynaceae	Leaves	Mianwali/March 2010	–	–	–	+	+	+	–	–	–	–	+	+	+	–
15	*Capparis decidua* (Forssk.) Edgew.	Karir	Capparaceae	Bark	Mianwali/March 2010	+	–	+	+	+	+	–	–	+	+	+	+	+	–
16	*Carthamus oxyacantha* M.Bieb.	Kandiari	Asteraceae	Flowers	Mianwali/March 2010	+	–	+	+	–	–	+	–	+	+	+	–	+	+
17	*Cassia fistula* L.	Amaltas	Fabaceae	Leaves	Shahdara/March 2010	+	–	+	+	–	–	–	–	+	+	+	+	–	–
18	*Catharanthus roseus* G. Don	Sadabahar	Apocynaceae	Leaves	Attock/March 2010	+	–	+	+	–	–	+	–	–	–	+	+	–	–
19	*Cedrela toona* M. Roem	Toon	Meliaceae	Leaves	Quaid-i-Azam university/April 2010	+	+	+	+	–	–	+	+	–	+	+	+	–	–
20	*Celosia cristata* L.	Kalgha, Cockscom	Amaranthaceae	Ariel parts	Sikandrabad(Mianwali)April 2010	+	–	+	–	–	–	+	+	–	+	+	+	–	–
21	*Centaurea calcitrapa* L.	Starthistle	Asteraceae	Ariel parts	Quaid-i-Azam university/April 2010	+	–	–	–	–	–	–	–	+	–	+	+	+	–
22	*Cicer arietinum* L.	Chick pea	Fabaceae	Leaves, Stem	Mianwali/March 2010	–	–	–	–	–	–	–	–	+	–	+	–	–	–
23	*Cirsium arvense* (L.) Scop.	Creeping thistle	Asteraceae	Stem	Khalabagh/April 2010	+	–	–	–	–	–	–	–	–	–	+	–	–	+
24	*Citrus limon* (L.) Burm.f.	Lemon	Rutaceae	Leaves	Malakwal/March 2010	–	–	–	+	–	–	–	–	–	–	+	–	+	+
25	*Colebrookea oppositifolia* Sm.	Dhusure	Labiatae	Leaves, flowers	Quaid-i-Azam university/April 2010	–	–	+	+	–	–	–	–	+	+	+	+	+	+
26	*Cordia myxa* L.	Lasura	Boraginaceae	Leaves	Mianwali/May-2010	–	–	+	–	–	–	–	–	–	–	–	–	–	+
27	*Coriandrum sativum* L.	Coriender	Apiaceae	Leaves	Mianwali/March 2010	+	–	+	–	+	–	–	–	–	–	–	–	–	–
28	*Cousinia minuta* Boiss	–	Asteraceae	Leaves	Kalabagh/January 2010	–	–	–	–	–	–	–	–	–	–	–	+	–	–
29	*Cuscuta reflexa* Roxb.	Amer bail	Convolvulaceae	Leaves, stem	Mianwali/March 2010	+	–	–	+	–	–	–	–	+	+	–	–	–	–
30	*Datura innoxia* Mill.	Downy thorn-apple	Solanaceae	Leaves, stem	Mianwali/March 2010	–	+	–	–	–	–	–	–	+	+	+	+	–	+
31	*Dodonaea viscosa* (L.) Jacq.	Hopbush	Sapindaceae	Leaves	Quaid-i-Azam university/April 2010	+	+	+	+	+	–	–	–	+	+	–	–	–	–
32	*Fagonia cretica* L.	Dhiman booti	Zygophyllaceae	Stem, spines	Hazara/May 2010	+	+	–	+	–	–	–	–	+	+	+	–	–	–
33	*Ficus microcarpa* L.fil	–	Moraceae	Leaves, bark	Quaid-i-Azam university/April 2010	+	+	–	+	+	–	–	–	–	–	–	–	–	–
34	*Fumaria indica* L.	–	Papaveraceae	Leaves Stem	Mianwali/March 2010	+	–	+	+	–	–	+	–	–	+	+	–	+	+
35	*Grewia asiatica* L.	Falsa	Malvaceae	Leaves	Mianwali/March 2010	+	+	–	+	–	–	–	–	–	–	–	–	+	+
36	*Ipomoea cornea* Jace.	Pink Morning glory	Convolvulaceae	Leaves	Bari imam/April 201	–	–	+	+	–	–	+	–	–	–	+	+	+	+
37	*Jasminum officinale* L.	Chambeli	Oleaceae	Leaves, stem	Mianwali/March 2010	+	–	–	+	+	+	–	–	+	+	–	–	–	–
38	*Jasminum Sambac* (L.) Aiton	Motia	Oleaceae	Leaves	Mianwali/March 2010	+	–	+	+	–	–	–	–	+	+	+	+	–	–
39	*Justicia adhatoda* L.	Arusa	Aanthaceae	Leaves	Bari imam/April 201	+	+	+	+	–	–	–	–	+	+	+	+	–	–
40	*Mallotus philippensis* Muell.	Kamala	Euphorbiaceae	Leaves	Shahdara/March 2010	+	+	+	+	+	+	–	–	+	+	+	+	+	+
41	*Mentha piperita* L.	Podina	Lamiaceae	Leaves, stem	Rawalpindi/April 2010	+	+	+	+	+	+	–	–	–	–	–	–	+	+
42	*Moringa oleifera* lam.	Drumstick tree	Moringaceae	Leaves, Bark	Kundian(Mianwali) March 2010	–	+	–	+	+	+	–	–	+	+	–	–	–	–
43	*Morus nigra* L.	Shiah toot	Moraceae	Leaves, Stem	Islamabad/March 2010	–	–	–	–	–	–	–	–	–	–	–	–	+	–
44	*Murraya koenigii* (L.) Spreng.	Curry tree	Rutaceae	Leaves	Salgira park/May 2010	–	+	–	–	–	–	+	–	+	+	+	+	–	–
45	*Nasturtium officinalen* W. T. Aiton	Watercresses	Brassicaceae	Leaves, stem	Salgira park/May 2010	–	–	–	–	–	–	–	–	+	+	–	–	+	–
46	*Nerium oleander* L.	Doghane	Apocynaceae	Leaves	Quaid-i-Azam university/April 2010	+	–	–	–	–	–	–	–	+	+	–	–	+	+
47	*Ocimum basilicum* L.	Tulsi	Lamiaceae	Ariel parts	Daudkha/April 2010	+	+	+	+	+	+	+	+	+	+	+	–	–	+
48	*Otostegia limbata* (Bth.)	Chittibooti	Lamiaceae	Leaves, stem	Lake view park/May 2010	+	–	+	–	+	–	+	–	–	–	+	+	–	–
49	*Peganum harmala* L.	Harmal	Nitrariaceae	Leaves, stem	Mianwali/March 2010	+	–	+	–	+	–	+	–	+	+	+	+	+	+
50	*Phyllanthus emblica* L.	Amla	Phyllanthaceae	Leaves	Quaid-i-Azam university/April 2010	+	+	+	+	+	+	+	–	+	+	–	–	+	+
51	*Pinus roxburghii* Sarg.	Chir pine	Pinaceae	Needles	Islamabad/March 2010	+	+	+	+	–	–	–	–	+	+	–	+	–	–
52	*Punica granatum* L.	Anar	Lythraceae	Leaves, Stem	Quaid-i-Azam university/April 2010	+	+	+	+	+	+	–	–	+	+	–	–	+	+
53	*Rhazya stricta* Decne.	Sehar,	Apocynaceae	Ariel parts	Musakhail /April 2010	+	–	–	–	–	–	–	–	–	–	–	+	–	–
54	*Ricinus communis* L.	Castor oil	Euphorbiaceae	Leaves	Islamabad/March 2010	+	+	+	+	–	–	–	–	+	+	+	+	–	–
55	*Rosa indica* L.	Gulab	Rosaceae	Leaves, stem	Islamabad/March 2010	+	+	+	+	+	+	–	–	–	–	+	+	–	–
56	*Rumex hastatus* L.	Khatti buti	Polygonaceae	Bark	Nathiagali/May 2010	–	+	+	+	–	–	–	–	+	+	+	+	–	–
57	*Sambucus nigra* L.	Elderberry	Adoxaceae	Leaves	Quaid-i-Azam university/April 2010	+	+	+	+	–	–	+	–	–	–	+	+	–	–
58	*Silybum marianum* (L.) Gaertn	Blessed milk thistle	Asteraceae	Ariel parts	Islamabad/March 2010	–	–	–	+	–	–	–	–	–	–	+	+	–	–
59	*Skimmia laureola* DC.	–	Rutaceae	Leaves	Quaid-i-Azam university/April 2010	+	–	+	–	–	–	–	–	+	–	+	–	–	–
60	*Syzygium cumini* (L.) Skeels	Jaman	Myrtaceae	Leaves	Mianwali/March 2010	+	+	+	+	+	–	–	–	+	+	+	–	+	+
61	*Withania coagulans* Dunal.	Ashwagandha	Solanaceae	Leaves, Stem	Mianwali/March 2010	+	+	–	+	–	–	+	+	+	+	+	+	–	–

+ = present; – = absent; a – methano/chloroform extract; b – aqueous extract.

2012b). Samples (100 μg/disc), two positive controls (Roxithromycin and Cefixime-USP, one for each) and negative control (DMSO) were used on each plate. Experiments were performed in triplicate and mean inhibitory zone was calculated with standard error.

2.4.2. Antifungal activity

Antifungal activity against five fungal strains (*Aspergillus flavus*, *Fusarium solani*, *Aspergillus fumigatus*, *Mucor* spp. and *Aspergillus niger*) was determined by the disc diffusion method (Abbasi et al., 2013). Sample concentration was 100 μg/disc and terbinafine (same concentration) was used as positive control. Petri dishes were incubated at 28 °C for 72 h and zones of inhibition were measured.

2.5. Antioxidant activities

2.5.1. Reducing power

The reducing power assay was performed according to the method described by Jafri et al. (2014). Concentration of each sample was 100 μg/mL and absorbance was measured at 630 nm using microplate reader (Biotech, Elx-800, USA). The reducing power of each sample was expressed as ascorbic acid (vit. C) equivalent.

2.5.2. Total antioxidant capacity (phosphomolybdenum method)

Total antioxidant capacity was determined by phosphomolybdenum method (Prieto et al., 1999). 0.1 mL of sample was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Mixture was incubated at 95 °C for 90 min and then cooled to room temperature Absorbance was measured at 695 nm. Antioxidant capacity of each sample was expressed as ascorbic acid equivalent.

2.5.3. DPPH (2,2-diphenyl1-1-picryl-hydrazyl radical) free radical scavenging

Free radical scavenging activities were determined as described by Bibi et al. (2011) with few modifications. 180 μl of DPPH solution (in methanol) was added to 20 μl of sample solution in DMSO (final concentration 100 μg/mL). Samples were incubated in dark at 37 °C for 15 min and absorbance was measured at 517 nm using microplate reader.

2.6. HPLC–DAD analysis of phenols and flavonoids

HPLC analysis of selected plant extracts (out of 61 plant species) was carried out using HPLC-DAD attached with Zorbax Rx-C8 analytical column using rutin, kaempferol, myricetin, gallic acid, catechin, caffeic acid and quercetin as standards. Method followed was as described by Jafri et al. (2014). Stock solution of each standard and sample was prepared at the concentration of 100 mg/ml and 10 mg/ml, respectively. Mobile phase A was methanol–acetonitrile–water–acetic acid (10:5:85:1) and mobile phase B was methanol–acetonitrile–acetic acid (60:40:1). A gradient of time 0–20 min for 0–50% B, 20–25 min for 50–100% B and then isocratic 100% B till 30 min was used. Flow rate was 1 ml/min and injection volume was 20 μl. Rutin and gallic acid were analyzed at 257 nm, catechin at 279 nm, caffeic acid at 325 nm and quercetin, myricetin, kaempferol were analyzed at 368 nm. Quantification was carried out by the integration of the peak using the external standard method.

2.7. Statistical analysis

Statistical comparisons were done by regression analysis using IBM SPSS Statistics version 19. Differences were significant at the level of $p < 0.01$. All the data are expressed as means ± standard deviation ($n = 3$).

3. Results and discussion

3.1. Phytochemical screening

3.1.1. Qualitative analysis of phytochemicals

Local inhabitant knowledge and literature about the curative properties helped for the selection of the plants under study. Detection of alkaloids, tannins and terpenoids in several extracts indicates that these were major secondary metabolites in these as shown in Table 1. These compounds are known to exhibit bioactive properties as triterpenoids display analgesic and anticancer properties (Ali et al., 2008). Saponins are reported to have hypocholesterolemic and antidiabetic properties, while triterpenoids display analgesic and anticancer properties (Ali et al., 2008). So these secondary metabolites contribute to potent use of plants in pharmacological industries. Differences were observed in current and previous studies. In current study, terpenoids, tannins, coumarins, saponins and cardiac glycosides were present in both M/C and aq. extracts of *Punica granatum* while alkaloids and quinones were not found. In previous studies alkaloids were found to be present in aq. extracts of the *Punica granatum* while terpenoids were not detected (Wadood et al., 2013). Similarly, in current study terpenoids and tannins were detected in *Ficus microcarpa* while in previous studies alkaloids and steroids have also been reported (Shripad et al., 2012). The differences were might be due to variation in genetic makeup, weather and geographic location of the plants and extraction procedure used or their phytochemicals. In our study M/C (1:1) proved to be a better solvent system for extraction of wide range of metabolites from these plants.

3.1.2. Quantitative analysis of phytochemicals

3.1.2.1. Total phenolic content (TPC). The results showed that the content of total phenols in extracts, expressed as gallic acid equivalents (GAE)/g dry weight (DW) of plant, varied to a great extent and ranged from 20.2 to 85.6 mg GAE/g DW in M/C extracts and 5.5–62.1 mg GAE/g DW in aq. extracts (Table 2). Similarly, Katalinic et al. (2006) while studying phenolic content of 70 medicinal plants observed a significant variation. We classified plant species into four categories due to wide range of TPC i.e. M/C extracts of 8 and aq. extracts of 6 plant species showed TPC more than 50 mg GAE/g DW, while M/C extracts of 35 and aq. extracts of 17 plants species had TPC between 30 and 49 mg GAE/g DW, TPC of 18 M/C extracts and 37 aq. extracts was between 10 and 29 mg GAE/g DW and none of the M/C extracts had TPC less than 10 mg GAE/g DW except aq. extract of one plant only i.e. *Silybum marianum*. Among all extracts, highest phenolic content was

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Table 2 Antioxidant potential and total phenolic and flavonoid content of M/C and Aq. extracts of 61 plants.

SN	Plant name	Reducing power assay[a] (Vit C equiv mg/g DW)		Total Antioxidant Capacity[a] (Vit C equiv mg/g DW)		DPPH Assay (%)				Total Phenolic Content [b](GAE/g DW)		Total Flavonoid Content [c](mg QE/g DW)	
		[d]M/C	[e]Aq.	M/C	Aq.	M/C	IC_{50}	Aq.	IC_{50}	M/C	Aq.	M/C	Aq.
1	*Ageratum conyzoides* L.	25.4 ± 2.1	40.6 ± 3.0	37.6 ± 1.5	20.9 ± 2.0	21.7 ± 1.3		29.0 ± 4.7		31.9 ± 2.0	26.5 ± 2.5	8.3 ± 2.2	12.8 ± 1.0
2	*Ajuga brateosa* Wall.	54.0 ± 2.0	25.8 ± 2.0	43.9 ± 2.6	16.2 ± 2.0	41 ± 1.5		40 ± 2.3		34.1 ± 3.0	29.0 ± 3.7	17.9 ± 2.0	19.0 ± 2.6
3	*Albizia labeek* L.	6.5 ± 1.0	9.6 ± 0.3	13.9 ± 1.5	6.5 ± 2.0	22.7 ± 1.4		15.6 ± 0.8		27.2 ± 2.5	15.0 ± 2.0	4.0 ± 4.1	6.5 ± 1.6
4	*Anagallis arvensis* Linn.	15.2 ± 2.0	18.7 ± 2.2	23.0 ± 4.0	17.9 ± 2.0	40.4 ± 3.1		30.8 ± 3.0		29.6 ± 1.0	25.6 ± 2.5	9.5 ± 2.1	12.6 ± 2.5
5	*Anethum graveolens* L. *vr.White flower*	25.7 ± 2.5	12.1 ± 2.5	15.1 ± 2.6	20.4 ± 5.0	18.4 ± 2.6		28.5 ± 2.5		29.7 ± 3.5	25.5 ± 1.0	11.9 ± 2.0	16.3 ± 1.8
6	*Anethum graveolens* L. *vr.yellow flower*	12.2 ± 0.4	27.4 ± 4.1	33.4 ± 1.6	22.8 ± 3.0	33.1 ± 2.5		3.9 ± 1.0		29.8 ± 2.0	27.3 ± 2.5	18.5 ± 1.6	8.9 ± 3.2
7	*Artemisia roxburghiana* Wall. ex Besser	26.1 ± 1.0	20.4 ± 4.0	44.2 ± 3.0	22.9 ± 1.0	38.5 ± 4.5		26.1 ± 2.5		30.3 ± 1.5	23.1 ± 2.5	12 ± 2.6	10.6 ± 5.0
8	*Azadirachta indica* A. Juss.	32.6 ± 2.5	34.4 ± 4.5	46.5 ± 1.0	9.7 ± 1.2	41.8 ± 4.0		6.8 ± 2.1		29.6 ± 4.5	27.2 ± 2.0	16.0 ± 2.5	14.2 ± 2.6
9	*Buxus papillosa* Schneider	16.6 ± 1.0	28.7 ± 2.0	31.8 ± 2.5	16.8 ± 1.5	41.2 ± 3.5		18.4 ± 2.5		31.3 ± 3.2	31.1 ± 3.0	10.5 ± 2.0	10.6 ± 2.1
10	*Berberis lycium* Royle	**80 ± 2**	45 ± 3.2	**87.2 ± 2.5**	24.4 ± 2.7	50 ± 2.8	**99.1**	30 ± 2.6		**45.1 ± 3.2**	26.2 ± 3.8	21 ± 2.6	8.4 ± 1.0
11	*Brassica campestris* L.	37.4 ± 1.0	19.7 ± 0.5	33.3 ± 1.0	9.2 ± 2.2	35.6 ± 2.5		20.2 ± 5.0		31.5 ± 5.0	26.6 ± 3.0	11.3 ± 2.1	7.8 ± 2.5
12	*Calendula arvensis* (Vaill.) L.	10.0 ± 1.0	12.1 ± 3.0	19.0 ± 3.2	25.2 ± 1.5	8.7 ± 0.6		48.7 ± 1.5		25 ± 2.0	24.6 ± 3.5	8.0 ± 2.3	10.2 ± 2.5
13	*Calendula officinalis* L.	9.1 ± 0.8	9.9 ± 2.8	14.4 ± 0.2	10.9 ± 1.0	26.4 ± 1.0		27.6 ± 2.0		27.0 ± 1.5	24.4 ± 2.0	7.5 ± 2.7	6.5 ± 1.0
14	*Calotropis procera* (Aiton) W.T. Aiton	15.5 ± 1.0	21.4 ± 2.5	28.3 ± 2.5	10.2 ± 2.0	35.7 ± 3.5		17.7 ± 2.0		29.1 ± 2.9	22.5 ± 3.0	12.1 ± 2.5	2.4 ± 0.2
15	*Capparis decidua* (Forssk.) Edgew.	19.6 ± 1.0	35.2 ± 4.5	30.4 ± 4.0	25.5 ± 2.0	40.5 ± 3.1		30.5 ± 1.5		30.3 ± 2.5	29.3 ± 3.5	21.9 ± 4.5	13.1 ± 3.0
16	*Carthamus oxyacantha* M.Bieb.	55.8 ± 2.0	45.3 ± 3.6	**51.4 ± 7.4**	28.4 ± 2.5	45.1 ± 4.0		33.7 ± 1.9		37.5 ± 1.6	29.2 ± 2.6	25 ± 2.6	15.8 ± 3.0
17	*Cassia fistula* L.	42.1 ± 1.1	30.9 ± 3.1	49.2 ± 4.0	20.9 ± 2.0	50.1 ± 0.6	**99.7**	25.8 ± 4.1		29.3 ± 5.0	29.1 ± 2.6	17.9 ± 2.5	12.6 ± 2.0
18	*Catharanthus roseus* G. Don	40.2 ± 2.0	36.2 ± 4.6	48.0 ± 2.5	27.5 ± 2.0	45.7 ± 3.4		30.0 ± 5.0		34.2 ± 3.5	30.8 ± 3.0	21.0 ± 3.5	11.4 ± 1.5
19	*Cedrela toona* M. Roem	39.4 ± 2.5	52.0 ± 4.7	50.7 ± 3.2	26.0 ± 1.5	45.1 ± 2.6		14.6 ± 1.0		34.1 ± 1.1	31.7 ± 1.5	25 ± 2.0	16.0 ± 1.5
20	*Celosia cristata* L.	13.5 ± 3.0	8.78 ± 1.0	14.3 ± 3.1	9.5 ± 3.0	21.1 ± 2.0		10.2 ± 5.5		30.5 ± 2.7	15.8 ± 2.2	8 ± 4.0	5.6 ± 1.0
21	*Centaurea calcitrapa* L.	23.9 ± 1.1	31.0 ± 1.5	34.7 ± 3.1	13.0 ± 2.0	35.4 ± 1.0		20.5 ± 2.5		35.1 ± 2.5	26.5 ± 2.5	14.6 ± 2.7	7.1 ± 1.0
22	*Cicer arietinum* L.	25.7 ± 2.2	26.6 ± 1.0	38.6 ± 4.2	9.2 ± 4.0	55.8 ± 2.2	**95.3**	15.6 ± 1.5		31.3 ± 2.5	20.3 ± 2.0	**25.4 ± 3.5**	10.8 ± 2.6
23	*Cirsium arvense* (L.) Scop.	41.1 ± 2.6	10.5 ± 1.0	17.4 ± 3.5	10.4 ± 2.5	30.2 ± 3.1		13.1 ± 1.1		20.8 ± 2.0	23.3 ± 2.1	23.9 ± 2.6	7.9 ± 2.0
24	*Citrus limon* (L.) Burm.f.	13.0 ± 1.5	15.6 ± 3.0	21.5 ± 2.5	16.1 ± 1.0	26.7 ± 1.6		26.0 ± 0.5		29.5 ± 5.0	30.9 ± 2.0	8.8 ± 3.8	13.6 ± 3.6
25	*Colebrookea* oppositifolia Sm.	27.4 ± 1.5	20.4 ± 4.0	23.3 ± 3.0	11.8 ± 2.5	55 ± 3.6	**80.5**	20.1 ± 3.5		32.3 ± 3.0	25.9 ± 2.1	9.9 ± 2.0	7.3 ± 1.6
26	*Cordia myxa* L.	15.7 ± 1.1	30.1 ± 2.5	20.8 ± 3.7	25.9 ± 3.7	30.7 ± 1.5		31.4 ± 1.0		30.7 ± 3.7	27.2 ± 2.0	12.1 ± 2.5	16.0 ± 2.0
27	*Coriandrum sativum* L.	16.2 ± 1.5	16.6 ± 1.5	30.2 ± 0.5	8.2 ± 1.8	45 ± 4.7		50 ± 1.5	**99.1**	27.5 ± 2.5	24.1 ± 3.1	11.2 ± 1.5	6.6 ± 1.5
28	*Cousinia minuta* Boiss	6.8 ± 1.5	4.9 ± 3.2	9.4 ± 1.6	11.2 ± 1.0	40.1 ± 2.5		34.6 ± 3.1		20.2 ± 2.9	23.7 ± 4.5	4.0 ± 2.0	8.1 ± 2.5
29	*Cuscuta reflexa* Roxb.	49.1 ± 2.0	46.1 ± 4.1	34.7 ± 4.4	31.9 ± 0.9	32.1 ± 7.9		30.2 ± 4.0		35 ± 1.73	36.6 ± 2.5	9.1 ± 3.4	8.3 ± 1.2
30	*Datura innoxia* Mill.	33.6 ± 1.07	20.4 ± 5.07	44.8 ± 1.0	11.6 ± 1.0	40.1 ± 3.5		27.5 ± 2.0		34.5 ± 3.1	31.7 ± 3.9	22.5 ± 3.0	11.7 ± 2.1
31	*Dodonaea viscosa* (L.) Jacq.	44.7 ± 0.5	40.2 ± 5.0	31.5 ± 4.2	34.6 ± 1.5	**71 ± 2.5**	**50.1**	55 ± 4.5	**95.1**	35.7 ± 1.6	38.2 ± 2.0	15 .0 ± 3.6	16.7 ± 2.0
32	*Fagonia cretica* L.	10.0 ± 1.1	20.5 ± 0.7	15.2 ± 2.0	15.1 ± 3.1	14.7 ± 1.5		14.5 ± 1.5		27.1 ± 2.50	29.4 ± 3.5	12.4 ± 4.0	11.7 ± 2.0
33	*Ficus microcarpa* L.fil	**84.0 ± 4.1**	**81.4 ± 3.0**	**88.6 ± 5.4**	**38.6 ± 3.1**	**75 ± 3.6**	**27.0**	**80 ± 5.0**	**19.0**	**49.4 ± 2.0**	**55.6 ± 3.7**	**40.0 ± 2.5**	**36.0 ± 1.1**
34	*Fumaria indica* L.	23.1 ± 1.7	29.1 ± 2.6	12.9 ± 2.5	30.8 ± 7.7	39 ± 5.0		36.3 ± 4.6		32.6 ± 2.5	23.0 ± 3.0	13.7 ± 2.5	12.9 ± 2.0
35	*Grewia asiatica* L.	17.1 ± 2.5	18.3 ± 4.1	23.9 ± 1.5	11.7 ± 1.0	16.3 ± 5.0		10.3 ± 1.0		30.2 ± 2.3	27.6 ± 1.5	2.9 ± 0.4	9.9 ± 0.5
36	*Ipomoea carnea* Jace.	46.4 ± 9.6	28.4 ± 2.1	40.6 ± 5.0	25.5 ± 1.0	34.0 ± 3.5		49.7 ± 2.8		39.2 ± 3.0	28.0 ± 2.5	30 ± 3.0	16.0 ± 2.0
37	*Jasminum officinale* L.	52.9 ± 2.0	50.7 ± 3.2	30.4 ± 4.5	**40.5 ± 2.0**	**78.3 ± 2.5**	**21.9**	**80 ± 4.1**	**15.7**	**40.0 ± 2.5**	**38.9 ± 2.8**	16.2 ± 1.6	**29.2 ± 2.1**
38	*Jasminum sambac* (L.) Aiton	9.5 ± 3.3	20.3 ± 1.0	16.8 ± 1.4	14.8 ± 2.1	32.6 ± 3.0		12.9 ± 1.5		27.0 ± 2.0	25.9 ± 2.6	6.6 ± 3.2	8.0 ± 2.0
39	*Justicia adhatoda* L.	49.8 ± 2.2	**55.2 ± 3.6**	48.5 ± 3.5	**49.5 ± 2.0**	50.1 ± 3.1	**98.2**	16.0 ± 2.0		**42.6 ± 3.5**	29.5 ± 4.5	20.1 ± 4.1	11.8 ± 2.5
40	*Mallotus philippensis* Muell.	**60.1 ± 2.5**	**59.6 ± 3.2**	**70.7 ± 3.7**	**39.9 ± 3.4**	**65 ± 4.0**	**47.5**	**68.0 ± 2.5**	**18.5**	**48.0 ± 3.5**	31.7 ± 3.5	21.5 ± 1.5	**20 ± 2.0**
41	*Mentha piperita* L.	**94.1 ± 1.5**	**115.0 ± 3.2**	**72.1 ± 3.0**	**50.8 ± 2.0**	**88.0 ± 6.1**	**23.0**	**80 ± 3.5**	**23.8**	**71.8 ± 5.1**	**55.1 ± 3.6**	**33.5 ± 2.5**	**37.1 ± 2.5**
42	*Moringa oleifera* lam.	19.6 ± 1.0	20.0 ± 1.7	18.70 ± 3.2	11.5 ± 2.5	26.6 ± 4.8		20.4 ± 5.0		30.2 ± 3.5	26.7 ± 0.8	6.9 ± 2.0	9.1 ± 3.5
43	*Morus nigra* L.	28.7 ± 1.0	37.2 ± 2.00	36.5 ± 2.0	15.6 ± 3.3	50 ± 3.6		45.0 ± 2.0		32.7 ± 1.6	33.6 ± 2.5	20.0 ± 2.6	21.9 ± 2.5
44	*Murraya koenigii* (L.) Spreng.	12.8 ± 1.1	23.3 ± 2.5	25.6 ± 2.0	22.0 ± 2.5	40.5 ± 4.0		31.5 ± 1.5		29.9 ± 2.0	38.5 ± 1.5	16.0 ± 2.0	12.1 ± 2.5
45	*Nasturtium officinalen* W. T. Aiton	11.1 ± 2.0	10.7 ± 2.0	15.9 ± 3.5	6.6 ± 2.1	20.0 ± 3.0		31.5 ± 1.6		27.9 ± 2.5	25.7 ± 2.5	9.3 ± 3.0	5.4 ± 1.6
46	*Nerium oleander* L.	**56.8 ± 2.0**	55.0 ± 5.0	48.6 ± 2.5	30.0 ± 2.5	49.4 ± 2.4		26.9 ± 1.0		32.5 ± 2.5	**46.0 ± 2.5**	**26.0 ± 2.0**	**33.3 ± 2.5**
47	*Ocimum basilicum* L.	45.4 ± 3.0	**59.0 ± 5.2**	35.2 ± 1.2	32.2 ± 2.5	55.0 ± 3.6	**91.0**	**70.0 ± 2.5**	**71.3**	33.7 ± 3.0	**39.8 ± 4.5**	6.9 ± 2.0	20.0 ± 1.5
48	*Otostegia limbata* (Bth.)	21.4 ± 2.0	22.7 ± 3.0	22.2 ± 1.6	10.4 ± 3.1	45.1 ± 2.6		39.3 ± 2.7		34.1 ± 3.0	28.0 ± 2.0	10.9 ± 1.1	15.5 ± 0.5
49	*Peganum harmala* L.	17.2 ± 1.0	20.2 ± 4.5	29.5 ± 3.1	9.9 ± 3.2	30.2 ± 3.2		16.4 ± 1.5		32.4 ± 3.01	24.3 ± 3.1	20.5 ± 2.0	5.5 ± 2.7
50	*Phyllanthus emblica* L.	**94.8 ± 2.8**	**80.0 ± 2.9**	**62.7 ± 3.5**	**55.9 ± 3.4**	**68.2 ± 4.0**	**5.8**	**65.4 ± 1.0**	**27.5**	**47.5 ± 1.3**	**41.8 ± 2.5**	**29.5 ± 2.7**	8.3 ± 1.0
51	*Pinus roxburghii* Sarg.	27.4 ± 1.5	**80.0 ± 4.0**	40.3 ± 3.1	**40.6 ± 3.5**	41.7 ± 3.5		**80.5 ± 7.0**	**57.9**	33.3 ± 3.0	55.4 ± 2.5	16.9 ± 2.0	25.1 ± 2.5
52	*Punica granatum* L.	**114.0 ± 4.5**	**123.9 ± 2.6**	**74.9 ± 3.1**	**57.0 ± 2.0**	**91.0 ± 3.2**	**6.2**	**89.0 ± 2.6**	**15.9**	**85.6 ± 3.3**	**62.1 ± 3.0**	**44.5 ± 3.1**	**35.9 ± 2.2**
53	*Rhazya stricta* Decne.	45.7 ± 1.0	30.5 ± 2.5	50.1 ± 3.7	31.1 ± 2.6	33.8 ± 3.0		15.1 ± 2.5		39.0 ± 5.0	34.2 ± 3.0	18.5 ± 2.5	14.2 ± 3.0
54	*Ricinus communis* L.	38.6 ± 1.6	39.8 ± 2.8	25.1 ± 0.5	19.2 ± 3.0	50.0 ± 4.0	**99.2**	33.1 ± 2.5		34.5 ± 3.1	29.3 ± 2.0	12.2 ± 2.1	15.0 ± 3.2
55	*Rosa indica* L.	**70.3 ± 3.1**	**72.7 ± 2.5**	**58.7 ± 2.5**	**50.2 ± 2.6**	**65.1 ± 4.0**	**9.0**	**61.7 ± 2.8**	**29.1**	**49.7 ± 3.5**	**41.1 ± 3.0**	**27.1 ± 2.0**	**24.7 ± 3.1**
56	*Rumex hastatus*L.	33.4 ± 1.5	26.1 ± 4.6	25.9 ± 2.0	18.6 ± 2.5	38.2 ± 3.1		31.0 ± 4.9		30.0 ± 3.2	28.2 ± 4.5	24.9 ± 3.0	10.6 ± 3.0
57	*Sambucus nigra* L.	**62.0 ± 2.5**	45.5 ± 1.0	**55.5 ± 3.3**	27.3 ± 2.7	**58.4 ± 2.1**	**91.2**	26.3 ± 2.0		36.5 ± 4.2	33.1 ± 3.0	**30.0 ± 3.6**	12.5 ± 2.5
58	*Silybum marianum* (L.) Gaertn	11.8 ± 1.2	8.0 ± 2.6	12.4 ± 1.3	5.8 ± 3.5	20.1 ± 0.6		25.9 ± 1.5		25.0 ± 7.0	5.5 ± 2.0	7.4 ± 1.6	5.9 ± 1.0
59	*Skimmia laureola* DC.	20.0 ± 1.7	21.2 ± 2.6	33.1 ± 0.0	15.2 ± 1.6	37.9 ± 1.9		35.9 ± 3.1		32.4 ± 2.5	35.1 ± 2.6	14.0 ± 2.0	10.1 ± 1.5
60	*Syzygium cumini* (L.) Skeels	**86.6 ± 3.0**	**108.0 ± 3.7**	**71.9 ± 5.1**	**56.7 ± 2.5**	**90 ± 4.5**	**8.2**	**89 ± 2.3**	**12.8**	**61.6 ± 3.0**	**60.2 ± 2.0**	**40.0 ± 2.5**	**21.3 ± 1.0**
61	*Withania coagulans* Dunal.	38.7 ± 2.2	25.7 ± 3.7	50.8 ± 4.3	18.3 ± 0.5	50.2 ± 2.0		31.9 ± 2.0		35.8 ± 3.7	27.4 ± 2.5	24.0 ± 2.6	12.6 ± 2.5

Experiment was performed in triplicate and data show the mean; ± = standard error.

[a] vit. C equivalent (eq.)/g DW.

[b] Gallic acid equivalents (GAE)/g dry weight (DW) of plant.

[c] Quercetin equivalents/g dry weight of the sample.

[d] Methanol/chloroform extracts.

[e] Aqueous extracts. Ten best values for each assay have been shown as bold numbers.

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recorded in M/C extract of *Punica granatum* (85.6 mg GAE/g DW) with maximum and diverse groups of phenolic compounds extracted in both the solvent systems used. Pharmacists usually target the plant with high phenolic content to treat different diseases (Petti and Scully, 2009). High amount of phenolic content indicates the ability of plant to treat inflammatory diseases and can be implicated in wound healing.

3.1.2.2. Total flavonoid content (TFC). TFC was calculated as quercetin equivalents (QE) ($Y = 0.0101x - 0.004$, $R^2 = 0.994$) as shown in Table 2. Flavonoids are important because of their ability to inhibit enzymes, anti-inflammatory activity and antimicrobial activity. The difference in TFC among studied plants varied significantly, ranging from 2.9 to 44.5 mg QE/g DW of sample for M/C extracts and 2.4–37.16 mg QE/g DW of plant for aq. extracts. We classified extracts of plant species into four categories i.e. 1st was of extracts with TFC more than 20 mg QE/g DW, 21 M/C and 11 aq. extracts fall in this category, 2nd with TFC between 15 and 20 mg QE/g DW, M/C extracts of 11 plants and aq. extracts of 9 plants have values in this range, 3rd with TFC between 10 and 15 mg QE/g DW, which included 13 M/C and 21 aq. extracts and last category was of extracts with TFC less than 10 mg QE/g DW, M/C extracts of 16 plants and aq. extracts of 20 plants had such a low flavonoid content. In current investigation highest TFC was found again in the M/C extract of *Punica granatum*.

3.2. Antimicrobial activities

3.2.1. Antibacterial activity

The plant extracts showed varying degree of antibacterial potential due to different chemical composition (Table 3). In general M/C extracts inhibited bacterial growth more effectively as compared to aq. extracts but interestingly aq. extracts of *Pinus roxburghii, Mentha piperita, Skimmia laureola* and *Morus nigra* inhibited the growth of *E. aerogens* more efficiently than their M/C extracts. It is also noteworthy that extracts of *Pinus roxburghii, Mentha piperita, Skimmia laureola, Mallotus philippensis, Phyllanthus emblica* and *Grewia asiatica* demonstrated broad spectrum antibacterial activity in both solvent systems. Data revealed that maximum inhibition in the growth of *S. aureus* was caused by the M/C extract of *Ageratum conyzoides* and aq. extract of *Grewia asiatica*. *Datura innoxia* (M/C extract) and *Pinus roxburghii* (aq. extract) significantly inhibited the growth of *B. subtilis*. *Datura innoxia* (M/C extract) also efficiently inhibited the growth of *E. coli*. *Mentha piperita* (M/C extract) was very active against *E. aerogens* and its aq. extract was very also potent against *E. coli, S. typhi, E. aerogens* and *B. bronchiseptica* (all gram negative bacteria). Our results indicated that *S. typhi*, *E. aerogens* and *B. bronchiseptica* were resistant to most of the extracts. This could be explained on the basis of presence of an extra outer membrane in their cell wall which resulted in selective permeability of samples but we also observed that extracts of *Pinus roxburghii, Centorea calcitropa, Mentha piperata, Skimmia laureola, Fagonia cretica, Mallotus philippensis, Coleobrookea oppositifolia, Phyllanthus emblica, Datura innoxia, Grewia asiatica* and *Ficus microcarpa* had ability to inhibit the above mentioned gram negative bacteria due to the presence of active compounds which can act by inhibiting the bacterial growth without necessarily penetrating into the bacterial cell itself (Mulaudzi et al., 2011). We noticed that alkaloids, saponins and flavonoids were detected in all extracts exhibiting antibacterial activities. Thus we deduce that these metabolites are responsible for their antibacterial activities. This is in agreement with reports of Jaberian et al. (2013) that plant extracts have antibacterial activities may be due to the presence of potent compounds such as flavonoids, alkaloids, tannins, etc. However, the absence of activities in extracts does not mean complete lack of bioactive compounds but might be due to the lower amount of these compounds or their actions were antagonized by the presence of other compounds. Roxithromycin (control 1) was found to be active against *B. subtilis*, *E. coli* and *Bordetella bronchiseptica* while cefixime (control 2) was active against all the strains except *E. coli*.

3.2.2. Antifungal activity

Results of the antifungal investigations are shown in Table 4. *Phyllanthus emblica* and *Anagallis arvensis* very strongly repressed the growth of all fungal strains used, so these plants are proposed for the isolation of compounds with remarkable antifungal properties. It is also worth noting that aq. extracts of *Azadirachta indica* and *Morus nigra* significantly inhibited the growth of *Mucor* spp. which is the major cause of mucormycosis. The results support the use of water extracts in traditional medicine as reported by Mulaudzi et al. (2011). Extracts of *Anethum graveolens vr. white flower, Peganum harmala, Mentha piperita, Cedrela toona, Justicia adhatoda, Citrus limon* and *Anethum graveolens vr. yellow flower* also expressed antifungal properties against few fungal strains. Wide variety of secondary metabolites are reported to exhibit antifungal properties especially flavonoids (Varghese et al., 2009). These metabolites were also detected in our samples exhibiting antifungal activity. Terbinafine was found to be very active against all the fungal strains with maximum zone of inhibition against *F. solani* (35.5 mm).

3.3. Antioxidant activities

3.3.1. Reducing power (RP)

Plants displayed a large variation in RP both in M/C and aq. extracts (Table 2) and the values ranged from 6.57 to 114 mg vit. C equivalent (eq.)/g DW in M/C extracts, with average value of 35.7 mg vit. C eq./g DW. The RP values for the aq. extracts ranged from 4.9 to 123.9 mg vit. C eq./g DW, with mean value 35.9 mg vit. C eq./g DW. Different RP values of plants and variable behavior of same plant in different solvents suggest that nature of plant and solvent system used for the extraction are very crucial to study antioxidant activity as observed by Qader et al. (2011) and Katalinic et al. (2006). Plants demonstrating remarkably high antioxidant activities in both the extracts include *Punica granatum*, *Phyllanthus emblica, Mentha piperita*, *Syzygium cumini*, *Ficus microcarpa*, *Rosa indica*. M/C extract of *Berberis lycium*, *Sambucus nigra* and *Mallotus philippensis* and aq. extract of *Pinus roxburghii* also showed remarkable RP. Due to their tremendous reduction capacity we recommend these plants for the extraction of antioxidant compounds for medicinal and commercial use. Aqueous extracts are mostly non-toxic and hence further isolation of antioxidant compounds is not necessary. In addition, due to synergistic effects of their phytochemical, health

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Table 3 Antibacterial activity against the tested gram positive and gram negative strains at 100 μg/disc concentration.

SN	Plant name[a]	Antibacterial activity of M/C extracts						Antibacterial activity of Aq. extracts					
		S. aur[b]	*B. sub*[c]	*E. coli*[d]	*S. typhi*[e]	*E. aero*[f]	*B. bron*[g]	*S. aur*[b]	*B. sub*[c]	*E. coli*[d]	*S. typhi*[e]	*E. aero*[f]	*B. bron*[g]
1	*Ageratum conyzoides*	19.5 ± 2.4	–	8.1 ± 0.5	–	–	–	–	–	–	–	–	–
2	*Ajuga brateosa*	6.8 ± 0.1	–	8.1 ± 0.6	–	–	–	–	–	–	–	–	–
3	*Anagallis arvensis*	–	–	9.2 ± 0.8	–	–	–	–	–	–	–	–	–
4	*Anethum graveolens vr.White flower*	9.4 ± 0.7	–	–	–	–	–	–	–	–	–	–	–
5	*Berberis lysium*	8.1 ± 0.5	–	10.4 ± 1.2	10.1 ± 0.4	–	–	–	–	–	–	–	–
6	*Capparis decidua*	8.4 ± 0.5	–	10.2 ± 0.9	–	–	–	–	–	–	–	–	–
7	*Cassia fistula*	13.2 ± 1.1	–	–	–	–	–	–	–	–	–	–	–
8	*Cedrela toona*	6.1 ± 0.4	–	10.2 ± 0.5	8.1 ± 0.1	–	–	–	–	–	–	–	–
9	*Celosia cristata*	7.2 ± 0.2	–	7.2 ± 0.5	–	–	–	–	–	–	–	–	–
10	*Centorea calcitropa*	8.2 ± 0.1	–	8.2 ± 0.4	14.5 ± 1.9	–	–	–	–	–	–	–	–
11	*Cicer arietinum*	10.3 ± 0.4	–	–	–	–	–	–	–	–	–	–	–
12	*Colebrookea oppositifilia*	8.1 ± 0.9	–	7.1 ± 0.2	–	6.1 ± 0.1	–	–	–	–	–	–	–
13	*Cuscuta reflexa*	–	–	–	–	–	–	–	–	–	8.2 ± 0.2	–	–
14	*Datura innoxia*	10.4 ± 1.2	13.1 ± 1.2	20.6 ± 2.5	–	11.6 ± 1.1	12.8 ± 1.5	–	–	–	–	–	–
15	*Dondonea viscosa*	–	7.0 ± 0.4	–	–	–	–	–	–	–	–	–	–
16	*Fagonia cretica*	11.2 ± 1.2	–	11.5 ± 1.2	–	9.2 ± 0.9	8.1 ± 0.3	–	–	–	–	–	–
17	*Ficus microcarpa*	9.2 ± 0.8	10.5 ± 0.9	–	8.1 ± 0.8	–	16.8 ± 1.9	–	–	–	–	–	–
18	*Grewia asiatica*	10.4 ± 1.1	–	–	15.2 ± 1.21	11.7 ± 1.3	6.1 ± 0.2	13.5 ± 1.6	–	–	–	–	–
19	*Mellotus philipinesis*	–	7.1 ± 0.11	10.2 ± 0.9	15.0 ± 1.2	8.2 ± 0.4	10.1 ± 0.8	–	8.4 ± 0.3	–	7.2 ± 0.5	10.5 ± 0.9	6.1 ± 0.05
20	*Mentha pierata*	10.1 ± 0.9	–	11.5 ± 1.2	–	14.2 ± 1.6	–	10.3 ± 0.7	–	15.4 ± 1.6	8.5 ± 0.7	16.5 ± 2.1	10.5 ± 0.7
21	*Morus nigra*	6.1 ± 0.5	–	6.1 ± 0.6	–	–	–	–	–	8.1 ± 0.4	–	10.8 ± 1.5	8.5 ± 0.4
22	*Ocimum basilicum*	–	–	–	–	–	–	8.8 ± 1.0	–	8.9 ± 1.5	–	–	–
23	*Otostegia limbata*	–	8.0 ± 0.1	11.3 ± 1.5	–	–	–	–	–	–	–	–	–
24	*Peganum harmala*	10.2 ± 1.5	–	–	–	–	–	–	–	–	–	–	–
24	*Phyllanthus emblica*	10.5 ± 1.3	–	11.3 ± 1.2	19.4 ± 2.3	12.5 ± 1.6	11.5 ± 1.3	–	–	–	–		9.5 ± 0.8
25	*Pinus roxburghii*	8.9 ± 0.4	8.1 ± 0.6	8.1 ± 0.4	–	6.1 ± 0.1	–	6.1 ± 0.6	10.3 ± 1.6	10.0 ± 1.1	–	13.1 ± 1.2	7.1 ± 0.1
26	*Punica granatum*	10.5 ± 0.8	8.2 ± 0.7	9.1 ± 0.1	–	–	–	8.5 ± 1.2	6.5 ± 0.1	–	–	–	–
27	*Rosa indica*	8.1 ± 0.6	9.1 ± 0.5	–	–	–	–	–	–	–	–	–	–
28	*Rumex hestatus*	10.5 ± 0.8											
29	*Silybum marianum*	–	–	9.5 ± 0.8	–	–	–	–	–	–	–	–	–
30	*Skimmia laureola*	–	–	8.5 ± 0.6	15.0 ± 1.5	11.5 ± 1.4	8.2 ± 0.1	–	–	–	–	12.5 ± 1.1	7.2 ± 0.01
31	*Syzygium cumini*	9.1 ± 0.8	10.0 ± 0.9	–	6.1 ± 0.1	–	–	–	–	–	–	–	–
	Roxithromycin	–	16.6 ± 1.2	30.8 ± 2.9	–	–	13.9 ± 1.2	–	15.8 ± 1.8	32.2 ± 2.9	–	–	14.2 ± 1.3
	Cefixime	20 ± 2.5	21.8 ± 2.9	–	21.5 ± 2.58	7.1 ± 0.6	15.4 ± 1.6	20.7 ± 2.9	22.7 ± 2.4	–	21.5 ± 2.2	7.5 ± 0.8	15.4 ± 1.6

[a] The plants which did not show activity against any of the bacterial strain were excluded from the above table. Test was performed in triplicate and data show the mean; ± = standard error.
[b] *S. aur*, *Staphylococcus aureus*.
[c] *B. sub*., *Bacillus subtilis*.
[d] *E. coli*, *Escherichia coli*.
[e] *S. typhi*, *Salmonella typhi*.
[f] *E. aero*, *Enterobacter aerogens*.
[g] *B. bron*, *Bordetella bronchiseptica*.

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Table 4 Antifungal activity against the tested strains at 100 µg/disc concentration.

SN	Plant name[a]	Antifungal activity of M/C extracts					Antifungal activity of Aq. extracts				
		A. fum[b]	*A. flav*[c]	*A. niger*[d]	*Mucor* sp.	*F. sol*[e]	*A. fum*[b]	*A. flav*[c]	*A. niger*[d]	*Mucor* sp.	*F. sol*[e]
1	*Anagallis arvensis*	20.5 ± 2.9	14.5 ± 1.8	12.5 ± 1.1	10.4 ± 1.2	18.5 ± 2.4	8.0 ± 0.7	–	–	–	–
2	*Anethum graveolens vr.White flower*	10.4 ± 0.9	–	8.0 ± 0.4	–	8.2 ± 0.2	–	–	–	–	–
3	*Anethum graveolens vr.yellow flower*	8.3 ± 0.5	–	–	–	10.4 ± 1.3	–	–	–	–	–
4	*Azadirachta indica*	–	–	–	–	–	–	6.0 ± 0.1	–	18.6 ± 1.8	–
5	*Citrus limon*	7.1 ± 0.6	–	–	–	–	–	–	–	–	–
6	*Justcia adhatoda*	8.1 ± 0.4	–	–	–	10.5 ± 1.1	–	–	–	–	–
7	*Mentha pierata*	8.2 ± 0.5	–	–	–	–	–	–	–	–	–
8	*Morus nigra*	–	–	–	–	–	–	6.2 ± 0.4	–	18.4 ± 2.4	–
9	*Peganum harmala*	7.1 ± 0.6	–	9.2 ± 0.6	–	8.1 ± 0.5	–	–	–	–	–
10	*Phyllanthus emblica*	20.6 ± 2.5	13.7 ± 1.5	11.0 ± 1.4	10.2 ± 1.0	15.7 ± 1.5	8.1 ± 0.6	–	–	–	–
	Terbinafine	30.6 ± 2.7	28.8 ± 1.9	33.5 ± 2.7	33.6 ± 2.9	35.5 ± 2.8	19.8 ± 2.1	25.3 ± 2.4	30.8 ± 2.8	30.6 ± 2.9	35.4 ± 2.8.

[a] The plant which does not showed activity against any of the bacterial strain has been excluded in the above table. Test was performed in triplicate and data show the mean; ± = standard error.
[b] *A. fum, Aspergillus fumigatus.*
[c] *A. flav, Aspergillus flavus.*
[d] *A. niger, Aspergillus niger.*
[e] *F. sol, Fusarium solani.*

benefits might be increased using plant infusions (Liu, 2003). Aq. extracts of most plants showed better RP than M/C extracts. Hence, it can be concluded that the compounds with reducing capacity are more efficiently extracted out by water and this agrees with the previous study of Wong et al. (2006) presenting the significance of aq. solvent for extracting reducing compounds.

3.3.2. Total antioxidant capacity (TAC)

Samples exhibited wide range of TAC values, expressed as the number of equivalents of ascorbic acid, from 9.4 to 88.6 mg vit. C eq./g DW in M/C extracts and 5.8–56.70 mg vit. C eq./g DW in aq. extract as shown in Table 2. *Ficus microcarpa* showed maximum TAC (88.64 µg vit. C eq./mg DW). None of aq. extract of any plant rose to category of highly significant TAC. This might be due to less extraction of phenolic compounds by aq. solvent which leads to decline in efficiency of these extracts to reduce Mo, this is in agreement with the report of Shon et al. (2004) who proposed a positive correlation between plant phenolic compounds and their antioxidant character.

3.3.3. DPPH free radical scavenging activity

The DPPH free radical scavenging activities were expressed as a percentage of inhibition and results are shown in Table 2. The results varied from 8 to 90% for M/C extracts and 3.9 to 89% for aq. extracts. Top plants exhibiting great antioxidant effects in both of the extracts were *Punica granatum, Syzygium cumini*, *Mentha piperita*, *Jasminum officinale*, *Ficus microcarpa*, *Dodonaea viscosa*, *Phyllanthus emblica*, *Rosa indica*, and *Mallotus philippensis*. It was noticed that the M/C and aq. extracts of *Punica granatum* showed the highest percentage scavenging i.e. 91% and 89%, respectively. From distribution of antioxidant activities, it appeared that most of the plant extracts have moderate DPPH values, either because of low concentration of the antioxidants and/or because of antagonistic behavior of the active compounds thus inhibiting the antioxidant effects. This is in accordance with the previous finding of Surveswaran et al. (2007) while working on 133 Indian medicinal plants.

Extracts with scavenging percentage more than fifty were selected for the determination of IC_{50} values. The IC_{50} value is defined as the concentration of plant extract with 50% free radical scavenging potential. Using three fold dilutions IC50 value of the M/C extract of *Phyllanthus emblica* was minimum among all the extracts while the M/C extract of *Punica granatum* also exhibited good IC_{50}. IC_{50} values of aq. extracts were found to be greater than M/C extracts with minimum value observed for *Syzygium cumini* i.e. 12.8 µg/ml.

3.4. Relationships among antioxidant capacity estimates made through reducing power, total antioxidant capacity and DPPH assay

Correlation analysis was performed to evaluate the suitability of the three assay methods used to determine the antioxidant activities of the 61 medicinal plants. Results are given in Fig. 1. Linear correlation (R^2) between RP and TAC was 0.75 while between RP and DPPH was 0.49 and between TAC and DPPH was 0.42 using M/C extracts. Almost similar linear correlation was observed between the assays performed

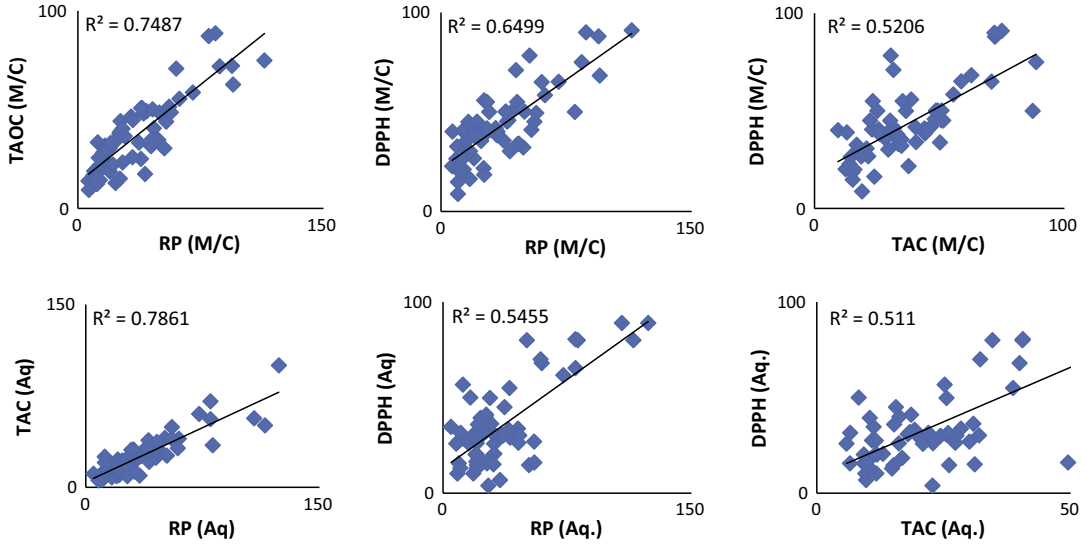

Figure 1 Correlation analysis between different antioxidant assays with respect to the solvent system. RP – Reducing Power, TAC – Total antioxidant, DPPH assays, R^2 – coefficient of determination. IBM SPSS Statistics version 19 was used to determine correlations. All results are significant at a level of $p < 0.01$.

using aq. extracts RP/TAC ($R = 0.78$), RP/DPPH ($R = 0.54$) and TAC/DPP ($R = 0.49$). These are not particularly strong correlations and differences attribute to different mechanism of action of antioxidant compounds in assay followed (Yildirim et al., 2000). In RP assay, antioxidants donate electrons to reduce ferric ion to ferrous ion while in TAC Mo (VI) is reduced to Mo (V) by the extracts at the acidic pH. As both the methods measure the reduction capacity of antioxidant compounds in the samples, the relationship was relatively strong. However, the slight differences observed might be due to different reaction conditions or/and difference in metal ions to be reduced. DPPH is a method based on the scavenging of the 1,1-diphenyl-2- picrylhydrazyl radical. Due to different mechanism of action of antioxidants in DPPH, the correlation of DPPH to RR and TAC was weak.

We observed that 38 plants exhibited much better antioxidant activity in TAC assay than RP assay while 44 plants showed enhanced DPPH activity than TAC and 2 plants showed same activity in DPPH and TAC while for 45 plants much better activity was observed in DPPH as compared to RP assay. From the methodological point of view the DPPH method is recommended as easy and accurate with regard to measuring the antioxidant activity of extracts. However, employing a method dependent on one mechanism may not reflect the true antioxidant capacity (Li et al., 2013). Hence, in the current research three types of antioxidant assays were performed to check the antioxidant potential of these plants.

The solvent system used for extraction plays a key role in assessing the antioxidant capacity of plants as the extractability of bioactive components depends on degree of polarity (Settharaksa et al., 2012). The data obtained revealed that the extracts of *Punica granatum*, *Ficus microcarpa*, *Mentha piperita*, *Phyllanthus emblica*, *Mallotus philippensis* and *Syzygium cumini* possess remarkable antioxidant potential exhibited in all the assays and in both the solvent systems. Therefore, these plants can be considered as a promising source of natural antioxidants.

3.5. Correlation between antioxidant activities TPC and TFC

Simple linear regression analysis was used to analyze the correlation between the antioxidant activities to TPC and TFC of 61 medicinal plants. The correlation coefficient (R^2) varied among different assays (Fig. 2). Relatively strong linear positive relationships were observed between the RP values to TPC (Fig. 2), which indicated the role of the phenolic compounds as reducing agents thus contributing to antioxidant activity. This result was in agreement with many reports (Li et al., 2013; Makhlouf et al., 2013). In this study slight correlation coefficient (R^2) was observed between TAC and TFC/TPC, but no correlation was observed between TAC and TFC of aq. extract i.e. $R^2 = 0.43$. Similarly, very weak linear correlation between DPPH free radicals scavenging percentage of sample to phenolic compounds might be due to different mechanism of action of this assay and nature of the plants under study as observed by Yu et al. (2002). However, from Table 2 it is apparent that few plants with high phenolic and flavonoid content have low antioxidant potential pointing to the difference in level of potency of various compounds. Contrarily, some plants with low phenolic and flavonoid contents showed significant antioxidant potential indicating that antioxidant activity of plant extracts is not limited to phenolic compounds but other secondary metabolites may also contribute to antioxidant potential (Javanmardi et al., 2003).

3.6. HPLC–DAD analysis of phenols and flavonoids

On the basis of antioxidant assays performed extracts of plants with high antioxidant potential were selected for quantitative analysis of highly potent known antioxidant compounds using reverse phase RP-HPLC fingerprinting. For this analysis standard antioxidants (rutin, kaempferol, myricetin, gallic acid, catechin, caffeic acid, apigenin and quercetin) were used as reference compounds. A RP-HPLC fingerprinting method was

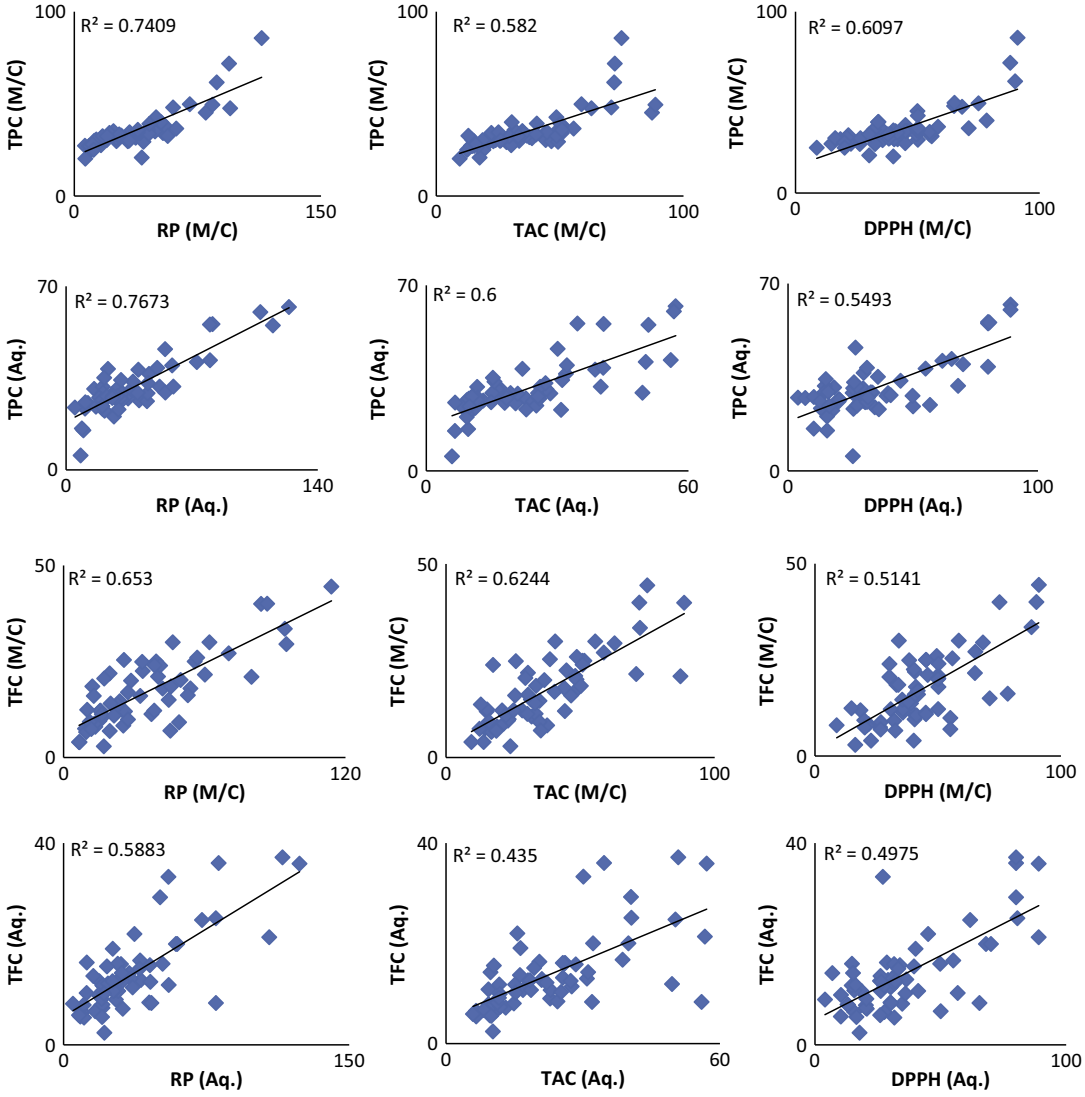

Figure 2 Correlations between of antioxidant activities of M/C an aq. extracts with TPC and TFC of 61 medicinal plants. Results are significant at $p < 0.01$ probability level. Where R^2 = coefficient of determination, M/C = methanol/chloroform extracts, Aq. = aqueous extracts, TPC = total phenolic content, TFC = total flavonoid content, TAC = total antioxidant capacity, RP = reducing power, DPPH = 2,2-diphenyl-1-picrylhydrazyl free radicle scavenging assay.

established for quantification of eight major polyphenols in the plant extracts which showed significant activities in all the assays. Results can be visualized in Table 5 and few representative chromatograms are shown in Fig. 3. Standards were selected on the basis of their reported medicinal properties for instance; gallic acid and catechin have antioxidant and anticancer properties (Zhao and Hu, 2013), caffeic acid reduces the acute immune and inflammatory response (Huang et al., 1998). Rutin has antihypertensive, antiviral and antiplatelet properties, as well as strengthening the capillaries, which is the result of its high radical scavenging activity and antioxidant capacity (Yang et al., 2008).

We found that the myricetin was the major antioxidant in M/C extracts of *Punica granatum*. In addition rutin and gallic acid were also detected while in aq. extracts gallic acid and catechin were the major antioxidants. Hmid et al. (2013) reported gallic acid, caffeic acid, catechin, quercetin and rutin in fruits extracts and myricetin was also reported in methanolic extract of pomegranate fruit by Naz et al. (2007). Gallic acid was found in majority of extracts except in the M/C extracts of *Jasminum officinale*, *Pinus roxburghhii* and *Dodonaea vicosa*. Therefore, it can be deduced that gallic acid is the major phenolic compound contributing to the antioxidant activity of the most of the plants. Maximum amount of gallic acid was detected in aq. extract of *Syzygium cumini*. It was noticed that in M/C extract of *Pinus roxburgii* only catechin was detected while in addition to catechin, gallic acid and apigenin were also observed in aq. extract which may be responsible for the enhanced antioxidant potential of the aq. extract.

3.7. Conclusion

The presented results offer supporting evidence for effective use of selected plant extracts. Plants naturally possess variety

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Table 5 Identification and quantification of the eight polyphenols in the M/C and aq. extracts of selected plants by RP-HPLC coupled with DAD.

Plant Names	Gallic acid µg/mg DW	Rutin µg/mg DW	Catechin µg/mg DW	Caffeic acid µg/mg DW	Apigenin µg/mg DW	Myricetin µg/mg DW	Quercetin µg/mg DW	Kaempferol µg/mg DW
Punica granatum (M/C)	3.38 ± 0.10	0.19 ± 0.05	–	–	–	4.75 ± 1.42	–	–
Punica granatum (Aq.)	3.61 ± .020	–	4.70 ± 2.0	–	–	–	–	–
Mallotus philippensis (M/C)	0.04 ± 0.05	0.33 ± 0.1	1.0 ± 0.8	–	–	–	0.11 ± 0.05	–
Mallotus philippensis (Aq.)	0.73 ± 0.10	–	–	0.10 ± 0.02	–	–	–	–
Phyllanthus emblica (M/C)	0.18 ± 0.02	0.19 ± 0.07	–	–	–	–	–	0.03 ± 0.01
Phyllanthus emblica (Aq.)	5.31 ± 0.20	0.78 ± 0.05	2.19 ± 1.50	0.01 ± 0.01	–	–	0.02 ± 0.01	–
Jasminum officinale (M/C)	–	0.11 ± 0.02	–	–	–	0.66 ± 0.20	0.78 ± 0.1	–
Jasminum officianle (Aq.)	0.06 ± 0.01	0.04 ± 0.01	–	–	–	–	–	–
Pinus roxburghii (M/C)	–	–	0.07 ± 0.04	–	–	–	–	–
Pinus roxburghii (Aq.)	0.03 ± 0.02	–	–	–	0.07 ± 0.01	–	–	–
Mentha pipperita (M/C)	–	–	–	0.03 ± 0.01	0.57 ± 0.10	4.28 ± 2.0	0.25 ± 0.08	–
Mentha pipperia (Aq.)	0.02 ± 0.01	0.03 ± 0.01	–	0.02 ± 0.01	0.15 ± 0.04	–	0.67 ± 0.4	–
Dodonaea viscosa (M/C)	–	0.05 ± 0.02	–	–	–	0.62 ± 0.44	–	0.03 ± 0.01
Dodonaea viscosa (Aq.)	0.03 ± 0.01	–	–	–	–	–	–	–
Rosa indica (M/C)	0.19 ± 0.05	–	0.75 ± 0.40	–	–	1.54 ± 0.40	–	–
Rosa indica (Aq.)	2.78 ± 1.10	–	1.00 ± 0.08	0.05 ± 0.01	–	2.61 ± 1.09	0.03 ± 0.01	–
Ficus microcarpa (M/C)	0.03 ± 0.01	0.03 ± 0.01	0.59 ± 0.20	–	–	–	–	–
Ficus microcarpa (Aq.)	0.08 ± 0.04	0.04 ± 0.02	0.89 ± 0.10	0.03 ± 0.01	–	0.64 ± 0.20	–	–
Syzygium cumini (M/C)	0.29 ± 0.06	0.03 ± 0.01	–	–	–	0.97 ± 0.40	0.05 ± 0.01	–
Syzygium cumini (Aq.)	5.20 ± 1.20	0.13 ± 0.04	2.50 ± 1.50	–	–	–	–	–

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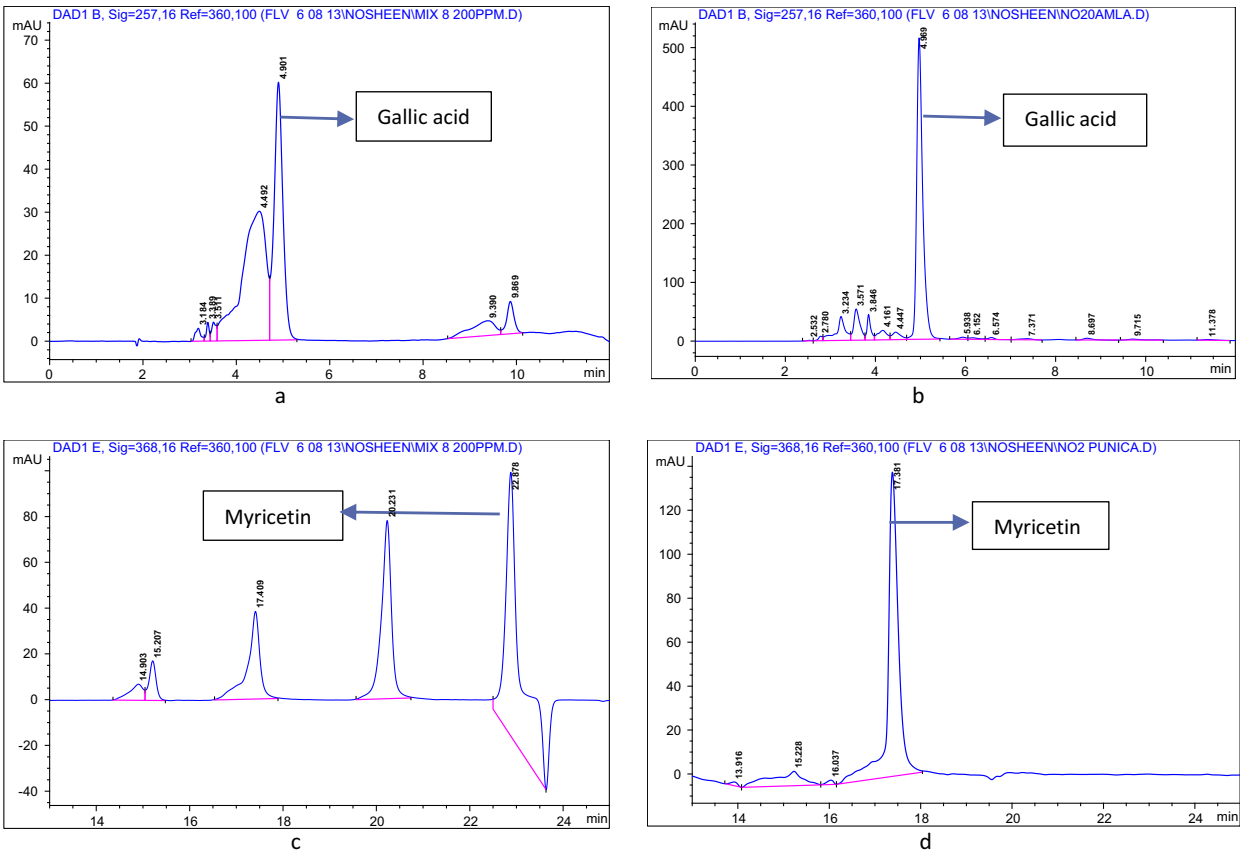

Figure 3 HPLC profile of (a) standard (gallic acid), (b) aq. Extract of *Phyllanthus emblica*, (c) standard (Myricetin) and (d) M/C extract of *Punica granatum*.

of therapeutic agents but properties depend on the nature of the plant, the system used to isolate these agents and method followed to evaluate the particular character. In the current study M/C extracts exhibited comparatively better activities in all the assays seemingly due to efficient extraction of phytochemicals. A significant relationship between the antioxidant capacities and phenolic compounds implied that these are the major contributors of antioxidant capacities of these plants. These results pave the way to isolate specific compounds with commercially valuable bioactive properties using appropriate plants on the basis of their respective therapeutic values and by selecting suitable solvent for extraction.

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