

Original article

Thin-layer chromatography and *in vitro* free radical scavenging activity of few medicinal plants from the surroundings of Junagadh, Gujarat, India

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Abstract

A collection of medicinal plants was done from the ambience of Junagadh district, Gujarat, India. These plants were subsequently screened to know their phytochemical potential. The *in vitro* antioxidant activity was also evaluated using DPPH and nitric oxide scavenging methods. Qualitative phytochemical screening and total phenolic content have been evaluated for each plant extract. TLC showed the presence of various phytochemicals like alkaloids, flavonoids, phenolics, glycosides, saponins, sterols and tannins. Methanol and water extracts of plants exhibited the highest amount of phenolic content. There was a difference in per cent inhibition of DPPH and nitric oxide scavenging activity showed by extracts. In the case of DPPH scavenging activity, water extracts of *Peltophorum pterocarpum* (DC.) K. Heyne leaves and *Syzygium cumini* (L.) Skeels leaves showed the highest free radical inhibition up to $76.37 \pm 0.42\%$ and $67.49 \pm 0.51\%$, respectively. In the case of nitric oxide scavenging action, all the extracts of *Bauhinia variegata* L. bark and *P. pterocarpum* leaves showed nitric oxide inhibition more than 75%. The plants like *B. variegata*, *P. pterocarpum* and *S. cumini* revealed high level of phenolic compounds. In conclusion, extracts of *B. variegata*, *P. pterocarpum* and *S. cumini* leaves can be served as the vital source of phenolics and flavonoids for having antioxidant potential.

Keywords: Antioxidant, DPPH, *in vitro* study, medicinal plants, nitric oxide, TLC

1. Introduction

Antioxidant compounds play a vital role as a health protecting factor. The primary source of these antioxidant compounds are fruits, vegetables and whole grains. The main role of antioxidant is to catalyze the simple redox reactions which occur in various biochemical processes by saturating lone-pair from free radicals, also called reactive oxygen species (ROS). These ROS are highly reactive and able to initiate many degenerative diseases such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer. Antioxidant compounds which mainly include polyphenols, phenolic acids, *etc.*, are commonly found in the plants (Brown and Rice-Evans, 1998; Scalbert *et al.*, 2005).

Human and other organisms of our body have an antioxidant defence system in the form of enzyme, metal chelating or free radical scavenging activities to prevent or reduce the ROS inducing oxidative damage. In addition, dietary antioxidants help to maintain the antioxidant status in the body. Antioxidants neutralize the free radicals by accepting or donating electron (s) to eliminate the lone pair of the radical. The antioxidant molecules are directly reacting

with reactive radical to destroy them and become new free radical itself, which are less reactive, less dangerous than the previous radical and longer-lived. Again, these radicals are neutralized by other free radical by a same or different mechanism and these reactions keep on going (Lü Jian-Ming *et al.*, 2010).

Although, synthetic antioxidants are available, their role has been criticized. Antioxidants obtained from natural sources, mainly from medicinal plants are very good agents. In the last few decades, the focus on natural antioxidant compounds has increased (Jayaprakash and Rao, 2000). Medicinal plants serve as a readymade source of the antioxidant compounds which are not harmful, economical and easily available. In India, it is estimated that 17,000 plant species exist and among these, 2000 species of medicinal plants are used by several ethnic communities in India for their medicinal properties (Mahendra *et al.*, 2016). Drugs of herbal origin provide a rational means for the treatment of several ailments in human and animals (Rajeshwari *et al.*, 2013; Manoharachary and Nagaraju, 2016; Nayanabhirama, 2016). A number of medicinal herbs are established antioxidants such as ginseng, curcuma, ginkgo, rosemary, green tea, grape, ginger and garlic, *etc.* They contain a wide variety of antioxidant compounds which include phenols, flavonoids, carotenoids, steroids and thiols (Lü Jian-Ming *et al.*, 2010). Potential role of herbal plants or phytomedicine in the area of oxidative stress has also been studied through *in vitro* or *in vivo* studies (Bhadarka *et al.*, 2018; Shaul *et al.*, 2018; Modi *et al.*, 2018).

There are many medicinal plants in and around Junagadh, Gujarat, India which may have an antioxidant potential. The present study

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was carried out for evaluation of the *in vitro* antioxidant potential of 10 medicinal plants using DPPH (2, 2-diphenyl-1-picrylhydrazyl) and nitric oxide scavenging methods along with phytochemical analysis through thin layer chromatography.

2. Materials and Methods

2.1 Collection and processing of plant material

All the plant materials as per Table 1 were collected from the

surroundings of Junagadh, Gujarat, India. Collected plant materials were identified and authenticated by Pharmacognosist. A specimen of each plant material was deposited in the Department of Veterinary Pharmacology and Toxicology, Veterinary College, JAU, Junagadh. Voucher specimen number of each plant is mentioned in Table 1. Collected plant materials were washed with tap water and dried in an oven at 45°C for 2 to 3 h. The material was ground; fine powder was made and stored in an air-tight container until use.

Table 1: List of medicinal plants used for evaluation of antioxidant activity

Sr. No.	Plant species (Specimen No.)	Family	Local name (Gujarati)	Part of plant used
1	<i>Adansonia digitata</i> L.(JVC/VPT/SP/PS/04/2015)	Bombacaceae	Gorakh ambali	Fruit
2	<i>Annona squamosa</i> L.(JVC/VPT/SP/PS/01/2014)	Annonaceae	Sitaphali	Leaves
3	<i>Bauhinia variegata</i> L.(JVC/VPT/SP/PS/05/2015)	Caesalpiniaceae	Kachanar	Bark
4	<i>Cassia tora</i> L.(JVC/VPT/SP/PS/07/2015)	Caesalpiniaceae	Kuwadiyo	Leaves
5	<i>Curcuma amada</i> Roxb.(JVC/VPT/SP/PS/06/2015)	Zingiberaceae	Amba haldar	Rhizomes
6	<i>Derris indica</i> (Lam.) Bennet (JVC/VPT/SP/PS/09/2015)	Fabaceae	Karanj	Seed
7	<i>Peltophorum pterocarpum</i> (DC.) K. Heyne (JVC/VPT/SP/PS/11/2015)	Caesalpiniaceae	Pilo gulmohar	Leaves and bark
8	<i>Punica granatum</i> L.(JVC/VPT/SP/PS/17/2015)	Punicaceae	Daadam	Epicarp
9	<i>Solanum xanthocarpum</i> Schrad. & H. Wendl. (JVC/VPT/SP/PS/18/2015)	Solanaceae	Bhoi ringani	Aerial part
10	<i>Syzygium cumini</i> (L.) Skeels (JVC/VPT/SP/PS/12/2015)	Myrtaceae	Kala jambu	Leaves

Table 2: Per cent yield and appearance of the different extracts of medicinal plants

Name of plant (Common names)	Extract	Appearance	% Yield
<i>Adansonia digitata</i> (Fruit)	CE	Green sticky mass	2.32
	ME	Green mass	4.88
	WE	Dark brown solid	10.60
<i>Annona squamosa</i> (Leaf)	CE	Green sticky mass	4.92
	ME	Brownish mass	7.92
	WE	Dark brown solid	8.24
<i>Bauhinia variegata</i> (Bark)	CE	Green sticky mass	1.80
	ME	Dark red mass	4.40
	WE	Red solid mass	13.00
<i>Cassia tora</i> (Leaf)	CE	Green sticky mass	3.48
	ME	Brownish mass	6.60
	WE	Dark brown solid	8.76
<i>Curcuma amada</i> (Rhizomes)	CE	Colorless sticky mass	1.16
	ME	Yellowish mass	3.72
	WE	Yellow solid mass	6.72
<i>Derris indica</i> (Seed)	CE	Colorless sticky mass	1.44
	ME	Yellowish mass	4.08
	WE	Light brown mass	7.96
<i>Peltophorum pterocarpum</i> (Leaf)	CE	Green sticky mass	1.56
	ME	Brownish mass	4.96
	WE	Dark brown	9.16
<i>Peltophorum pterocarpum</i> (Bark)	CE	Colorless sticky mass	1.00
	ME	Dark red mass	6.56
	WE	Dark red solid	15.16
<i>Punica granatum</i> (Epicarp)	CE	Green sticky mass	2.88
	ME	Brownish mass	6.52
	WE	Dark brown	7.80
<i>Solanum xanthocarpum</i> (Aerial part)	CE	Green sticky mass	2.04
	ME	Brownish mass	6.16
	WE	Dark brown	8.40
<i>Syzygium cumini</i> (Leaf)	CE	Light green sticky mass	2.12
	ME	Brownish mass	8.40
	WE	Dark brown	9.60

CE=chloroform extract, ME= methanol extract, WE= water extract.

2.2 Preparation of extracts

Fine powder of each plant material was defatted using n-hexane by Soxhlet apparatus to remove chlorophyll and other non-polar debris. The defatted plant material was dried in the oven below 45°C. About 50 g of plant material was extracted with 500 ml of chloroform, methanol and water separately at least two times. The content was filtered off and solvents were evaporated under reduced pressure using a rotary vacuum evaporator. The extracts were collected; yield was calculated and stored at 4°C for further use.

2.3 Phytochemical screening

Qualitative phytochemical screening was performed for each extract as per standard procedures (Harborne, 1998). Per cent yield and appearance of each plant extracts are shown in Table 2.

2.4 Total phenolic content

Total phenolic content was also measured by Folin Ciocalteu method (Encarnação *et al.*, 2015). In this assay, 250 µl of extract solution was allowed to react with 2 ml Folin-Ciocalteu (FC) reagent (previously diluted 1:10) and 1 ml solution (75 g/l) of sodium carbonate. The mixture was allowed to stand for 1 to 2 h. Absorbance was recorded at 760 nm in a spectrophotometer (Fusion Tek UV2900). Tests were performed in triplicate. Total phenolic content (TPC) was expressed as milligrams of gallic acid equivalent (GAE).

2.5 Thin layer chromatography (TLC) of plants

Thin layer chromatography (TLC) was performed to evaluate the presence of various phytochemicals in the different medicinal plant extracts used for the evaluation of an *in vitro* antioxidant activity. The TLC of each plant extract was performed on 10 × 10 cm pre-coated aluminium-backed silica gel plates GF₂₅₄ (Merck, Darmstadt, Germany). The samples were applied as a band using Linomat 5 applicator (Camag, Germany).

2.5.1 Detection of alkaloids

About 2 g of powdered plant material of *A. squamosa* leaves, *C. amada* rhizomes, *S. xanthocarpum* aerial part, and *S. cumini* leaves was moisten with 1 ml of strong ammonia and mixed thoroughly with mortar and pestle for 2 min. Then, 7 g of silica gel (60-120 mesh) was added to this mass to get uniform cohesive mass. This mass was packed loosely in a glass column 2 cm × 30 cm. The column was eluted with 25 ml of chloroform. The chloroform fraction collected in a beaker was evaporated to dryness. The dried mass was dissolved in methanol and used as samples. The plate was allowed to run in the pre-saturated mobile phase consist of toluene: ethyl acetate: diethylamine (7:2:1). After development, the plates were observed in the UV chamber to detect the presence of alkaloids (Wagner and Baldt, 1996) and dipped in drangendorff's reagent for the visualization of bands.

2.5.2 Detection of glycosides

Thin layer chromatography of glycosides was carried out as per the method given by Lokesh *et al.* (2017). Briefly, one gram of each dried plant material listed in Table 3 was extracted with 70% methanol by heating at 60°C for 10 min. After cooling, the content was centrifuged to 2500 rpm for 10 min. Each supernatant was used as a sample for the detection of glycosides. The TLC plate was developed in a pre saturated mobile phase consist of ethyl

acetate: methanol: water (20:2.5:2.5). The plate was observed in UV chamber at 366 nm light after spraying with Carr-Price reagent (20% antimony (III) chloride in chloroform). The glycoside shows blue to violet fluorescence.

2.5.3 Detection of saponin

Dried plant material of *A. digitata* fruit, *A. squamosa* leaves and *S. cumini* leaves were extracted with 70% methanol with heating at 60°C for 10 min. The content was centrifuged and the supernatant was dried, re-suspended in water containing a small amount of methanol, and shake with n-butanol (pre-saturated with water). An n-butanol layer was collected and used as TLC samples for the detection of saponin. The mobile phase consists of chloroform: glacial acetic acid: methanol: water (64:32:12:8). After development, the plate is sprayed with Carr-Price reagent (20% antimony (III) chloride in chloroform). The saponin exhibits blue fluorescence in UV 365 (Wagner and Baldt, 1996; Waksmundzka-Hajnos, Sherma and Kowalska, 2008).

2.5.4 Detection of tannins

TLC of plant material for detection of tannins was carried out as per the method given by Helen *et al.* (2015). About 1 g of fine powder of each *B. variegata* bark, *P. pterocarpum* bark and *P. granatum* epicarp was extracted with 10 ml of 70% acetone solution in 15 ml polypropylene tubes. After vigorous shaking and sonication, the tubes were centrifuged at 2500 rpm for 10 min to get the clear supernatant. The volume was reduced to 1/3 and used as samples. The samples were applied to the TLC plates as bands. The plate was developed in mobile phase consist of toluene: acetone: formic acid (60:60:10) pre-saturated for 30 min. Gallic acid (0.5 mg/ml in methanol) and tannic acid (0.5 mg/ml in methanol) were used as reference standard compounds. After development, the plates were sprayed with a 3% methanolic ferric chloride solution. The spots turn into dark bluish black against a light yellow background in daylight (Richter, 2002).

2.5.5 Detection of sterols

TLC of *S. xanthocarpum* aerial part and *B. variegata* bark for detection of phytosterol was carried out. One gram plant powder of the plants was extracted with 10 ml of 9:1 v/v mixture of petroleum ether: methanol in a beaker using sonication for 10 min. After sonication, the extract was filtered off and evaporated to dryness. The residues left over were dissolved in methanol and used as samples. The elution was carried out in n-hexane: diethyl ether (7:3 v/v). After development, the plate was sprayed with 50% methanolic sulfuric acid. After drying of the plates, the plates were observed in the UV chamber at 366 nm.

2.5.6 Detection of flavonoids

The plant extracts for the detection of flavonoids were prepared by extracting 2 g of each plant material in 10 ml of methanol on ultrasonic bath for 10 min. Then, the extracts were centrifuged to 2500 rpm for 10 min, supernatants were collected and used as a sample. Both quercetin (Sigma-Aldrich, India) and rutin (SD Fine Ltd, India) were dissolved in methanol at a concentration (0.5 mg/ml).

Two different solvent systems were used for the detection of quercetin and rutin and related phytochemicals in the plant extracts, *viz.*, ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26) for the detection of rutin (Wagner and Bladt, 1996)

while n-hexane: ethyl acetate: glacial acetic acid (31:14:5) for the detection of quercetin (Medic-Saric *et al.*, 2004) and related compounds in each plant extract. The samples were applied from 1 cm from the edge of the plate and allowed to run up to 8 cm. Derivatization was achieved by spraying natural product reagent (1% diphenyl boryloxyethylamine in methanol followed by 5% polyethylene glycol-4000 in methanol). The plates were then observed in the UV cabinet (Camag, Germany) at 366 nm.

2.5.7 Detection of phenolic compounds

The presence of phenolic compounds with special reference to gallic acid was detected with TLC as per the method is given by Shah *et al.* (2016). The plant powder (1 g) of each plant was extracted with 8 ml methanol on sonication for 10 min. After sonication, the content was centrifuged and the supernatant was collected, reduced the volume to 1/3, and used as samples. The chromatographic separation was achieved in the mobile phase consists of toluene: ethyl acetate: formic acid: water (6:6:1.2:0.25). After the development of bands up to 8 cm, the plates were dried and sprayed with natural product reagent (1% diphenylboryloxyethylamine in methanol followed by 5% polyethylene glycol-4000 in methanol). The plates were then observed in the UV cabinet (Camag, Germany) at 366 nm.

The R_f values (Retention factor) of each separated bands and standard compounds were calculated using dividing distance travelled by each solute to the total solvent front (8 cm).

2.6 Antioxidant activity

2.6.1 DPPH scavenging activity

Three ml of each extract concentrations/ascorbic acid solution were taken in the glass test tubes. One ml of 0.1 mM DPPH solution (4 mg in 100 ml methanol) was added. The solution was kept for 30 min at room temperature until the colour changed from violet to yellowish violet to yellow. Absorbance at 517 nm in spectrophotometer was recorded. A blank solution containing distilled water and DPPH alone (Control) were also prepared and spectra were recorded for control purpose. Ascorbic acid was used as the standard antioxidant compound. All tests were performed in triplicate (Senguttuvan *et al.*, 2014).

2.6.2 Nitric oxide scavenging activity

Three ml of all concentrations of prepared extracts were added with 500 μ l of 10 mM sodium nitroprusside solution and kept for 3 h. The mixture was added with 250 μ l Griess reagent (1% sulphanimide, 2% orthophosphoric acid, 0.1% N-1-naphthylethylenediamine di-hydrochloride [NED]) which produced pink colour in the solution. The mixture was allowed to stand for 30 min at room temperature and the absorbance at 546 nm in spectrophotometer was recorded. Antioxidant activity of both the assays was measured by calculating % inhibition against a range of concentrations. % inhibition can be calculated as follow: % inhibition = $(1 - Ac/At) \times 100$; where *Ac* is absorbance of control; *At* is absorbance of the test. Ascorbic acid was used as standard antioxidant compound (Senguttuvan *et al.*, 2014).

2.7 Statistical analysis

All the data are represented as Mean \pm SE. Data DPPH and nitric oxide scavenging activity were analyzed by one-way analysis of variance (one-way ANOVA), followed by Duncan Multiple Range Test (DMRT).

3. Results

Phytochemical screening was performed as per standard methods and various phytochemicals detected by chemical methods. Extracts of *A. squamosa* leaf, *C. amada* rhizome, *S. cumini* leaf and *S. xanthocarpum* aerial part showed presence of alkaloids. Extracts of *B. variegata* bark, *P. granatum* fruit epicarp and *P. pterocarpum* bark were found with presence of tannins. Flavanoids were detected in extracts of *A. digitata* fruit, *A. squamosa* leaf, *B. variegata* bark, *C. amada* rhizome, *C. tora* leaf, *D. indica* seed, *P. pterocarpum* leaf, *P. pterocarpum* bark, *P. granatum* fruit epicarp, *S. cumini* leaf and *S. xanthocarpum* aerial part (Table 3). Methanol and water extract of *B. variegata* (bark), *P. pterocarpum* (leaf and bark), *P. granatum* (epicarp) and *S. cumini* (leaf) were found with high total phenolic content (Table 4). Comparatively, methanolic and water extract of *P. pterocarpum* (leaf and bark) showed higher phenolic content about 47 and 50 mg GAE per gram, respectively. Different R_f values of alkaloid detected are shown in Table 5 and Figure 1.

Table 3: Phytochemical screening of different extracts of medicinal plants

Sr. No.	Name of phytochemical	Name of medicinal plants
1.	Alkaloid	<i>A. squamosa</i> leaf extract, <i>C. amada</i> rhizome extract, <i>S. cumini</i> leaf extract, <i>S. xanthocarpum</i> aerial part extract
2.	Glycoside	<i>A. digitata</i> fruit extract, <i>B. variegata</i> bark extract, <i>C. amada</i> rhizome extract, <i>C. tora</i> leaf extract, <i>D. indica</i> seed extract, <i>P. pterocarpum</i> leaf extract, <i>P. pterocarpum</i> bark extract, <i>P. granatum</i> fruit epicarp extract, <i>S. xanthocarpum</i> aerial part extract
3.	Saponin	<i>A. digitata</i> fruit extract, <i>A. squamosa</i> leaf extract, <i>S. cumini</i> leaf extract
4.	Tannin	<i>B. variegata</i> bark extract, <i>P. granatum</i> fruit epicarp extract, <i>P. pterocarpum</i> bark extract
5.	Sterol	<i>B. variegata</i> bark extract, <i>S. xanthocarpum</i> aerial part extract
6.	Phenolic compounds	<i>A. digitata</i> fruit extract, <i>A. squamosa</i> leaf extract, <i>B. variegata</i> bark extract, <i>C. amada</i> rhizome extract, <i>C. tora</i> leaf extract, <i>D. indica</i> seed extract, <i>P. pterocarpum</i> leaf extract, <i>P. pterocarpum</i> bark extract, <i>P. granatum</i> fruit epicarp extract, <i>S. cumini</i> leaf extract, <i>S. xanthocarpum</i> aerial part extract
7.	Flavonoids	<i>A. digitata</i> fruit extract, <i>A. squamosa</i> leaf extract, <i>B. variegata</i> bark extract, <i>C. amada</i> rhizome extract, <i>C. tora</i> leaf extract, <i>D. indica</i> seed extract, <i>P. pterocarpum</i> leaf extract, <i>P. pterocarpum</i> bark extract, <i>P. granatum</i> fruit epicarp extract, <i>S. cumini</i> leaf extract, <i>S. xanthocarpum</i> aerial part extract.

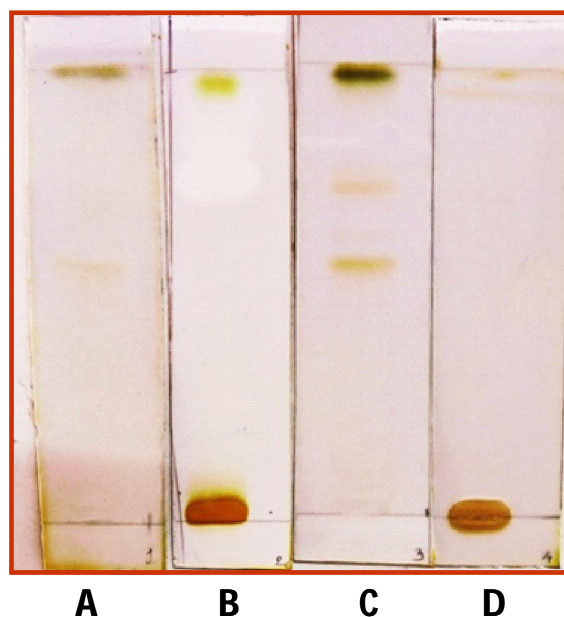
Table 4: Total phenolic content (mg GAE per gram) of different extracts of medicinal plants

Name of plant	Extract	Total phenolic content (mg GAE per gram)
<i>Adansonia digitata</i> (Fruit)	CE	4.89 ± 0.01
	ME	2.54 ± 0.11
	WE	0.08 ± 0.21
<i>Annona squamosa</i> (Leaf)	CE	1.61 ± 0.17
	ME	7.87 ± 0.23
	WE	2.58 ± 0.08
<i>Bauhinia variegata</i> (Bark)	CE	3.05 ± 0.08
	ME	47.41 ± 5.29
	WE	36.42 ± 0.54
<i>Cassia tora</i> (Leaf)	CE	4.89 ± 0.11
	ME	8.99 ± 0.14
	WE	4.68 ± 0.17
<i>Curcuma amada</i> (Rhizomes)	CE	9.75 ± 0.10
	ME	2.92 ± 0.19
	WE	2.58 ± 0.08
<i>Derris indica</i> (Seed)	CE	1.04 ± 0.10
	ME	3.19 ± 0.08
	WE	2.09 ± 0.14
<i>Peltophorum pterocarpum</i> (Leaf)	CE	7.50 ± 0.07
	ME	41.98 ± 0.11
	WE	72.73 ± 0.50
<i>Peltophorum pterocarpum</i> (Bark)	CE	6.32 ± 0.12
	ME	47.22 ± 0.15
	WE	50.53 ± 0.08
<i>Punica granatum</i> (Epicarp)	CE	1.90 ± 0.11
	ME	29.19 ± 0.18
	WE	38.62 ± 0.01
<i>Solanum xanthocarpum</i> (Aerial part)	CE	1.98 ± 0.16
	ME	4.90 ± 0.12
	WE	5.98 ± 0.11
<i>Syzygium cumini</i> (Leaf)	CE	2.02 ± 0.13
	ME	36.47 ± 0.09
	WE	16.59 ± 0.24

CE=chloroform extract, ME= methanol extract, WE= water extract, GAE= Gallic Acid Equivalent

Table 5: R_f values of alkaloids detected in plants

Name of extract/plant	R _f values
<i>A. squamosa</i> (Leaf)	0.57, 0.97
<i>C. amada</i> (Rhizome)	0.03, 0.98
<i>S. cumini</i> (Leaf)	0.58, 0.75
<i>S. xanthocarpum</i> (Aerial part)	0.03, 0.99

**Figure 1:** Detection of alkaloids by TLC in plants; A = *A. squamosa* leaves; B = *C. amada* rhizome; C = *S. cumini* leaves; D = *S. xanthocarpum* aerial parts.

TLC of various plant extracts exhibited the presence of glycosides, saponins, tannins, phytosterol, phenolic compounds and flavonoids. R_f values of glycosides, saponins, tannins, phytosterol, phenolic compounds and flavonoids are shown in Table 6 to Table 11, respectively. Detected glycosides, saponins, tannins, phytosterol and phenolic compounds are shown in Figure 2 to 6, respectively. *P. pterocarpum* (leaf), *P. pterocarpum* (bark), *P. granatum* (fruit epicarp), *S. cumini* (leaf) and *S. xanthocarpum* (aerial part) exhibited the presence of gallic acid with R_f value of 0.52. The *C. tora* (leaf) extract was found with presence of quercetin with R_f value of 0.23 with the solvent system of n-hexane: ethyl acetate: glacial acetic acid (31:14:5). Rutin was also detected in *C. tora* (leaf) and *A. squamosa* (leaf) extract with R_f value of 0.53 with the solvent system of ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26) (Table 11, Figure 7 and 8).

Table 6: R_f values of glycosides detected in plants

Name of extract/plant	R _f values
<i>A. digitata</i> (Fruit)	0.025, 0.53, 0.58
<i>B. variegata</i> (Bark)	0.025
<i>C. amada</i> (Rhizome)	0.025, 0.21, 0.31, 0.38, 0.5, 0.66
<i>C. tora</i> (Leaf)	0.025, 0.65, 0.9
<i>D. indica</i> (Seed)	0.025, 0.91
<i>P. pterocarpum</i> (Leaf)	0.65, 0.9
<i>P. pterocarpum</i> (Bark)	-
<i>P. granatum</i> (Fruit epicarp)	0.63, 0.71
<i>S. xanthocarpum</i> (Aerial part)	0.16, 0.38, 0.72

Table 7: R_f values of saponin detected in plant extracts

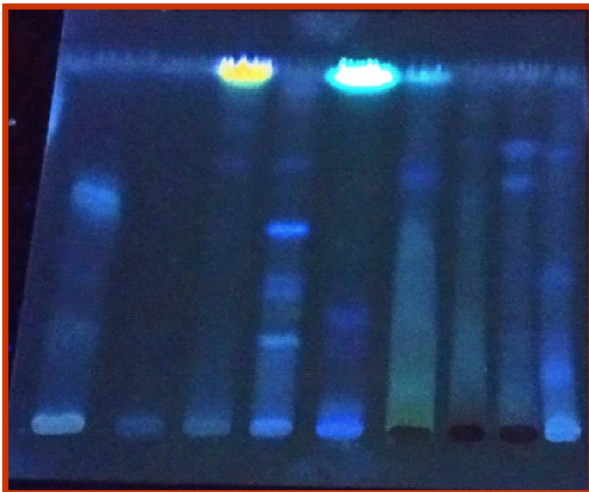
Name of extract/plant	R_f values
<i>A. digitata</i> (Fruit)	0.13, 0.28, 0.47, 0.66
<i>A. squamosa</i> (Leaf)	0.17, 0.23, 0.26, 0.36, 0.42, 0.58, 0.63, 0.72, 0.82
<i>S. cumini</i> (Leaf)	0.35, 0.53, 0.63, 0.73, 0.78, 0.82, 0.9

Table 8: R_f values of tannin detected in plant

Name of extract/standard	R_f values
<i>P. granatum</i> (Fruit epicarp)	0, 0.22, 0.7
Gallic acid	0.7
Tannic acid	0, 0.22, 0.26, 0.7

Table 9: R_f values of phytosterol detected in plant

Name of extract/plant	R_f values
<i>B. variegata</i> (Bark)	0.95
<i>S. xanthocarpum</i> (Aerial part)	0.13, 0.23, 0.95



A B C D E F G H I

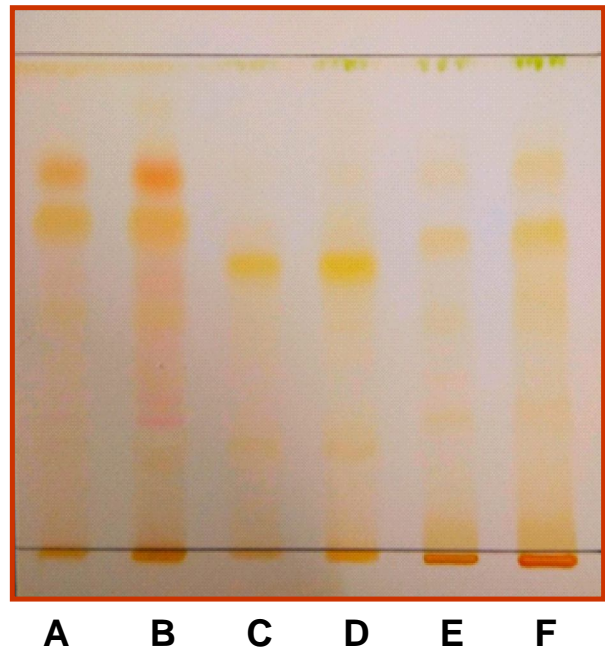
Figure 2: Detection of glycosides by TLC in plants; A = *A. digitata* fruit; B = *B. variegata* bark; C = *C. tora* leaf; D = *C. amada* rhizome; E = *D. indica* fruit; F = *P. pterocarpum* leaf; G = *P. pterocarpum* bark; H = *P. granatum* fruit epicarp; I = *S. xanthocarpum* aerial part.

Water extract of *P. pterocarpum* leaves, water extract of *B. variagata* bark, water extract of *S. cumini* leaves and methanol as well as chloroform extract of *S. cumini* leaves showed more DPPH scavenging activity as compared to extracts of other plant materials used in the study (Figure 9). Chloroform extract of *B. variagata* bark, water extract of *B. variagata* bark, methanol extract of *B. variagata* bark where all types of extracts of *P. pterocarpum* leaves showed more nitric oxide scavenging activity compared to other extracts used in the study (Figure 10 and 11).

Table 10: R_f values of phenolic compounds detected in plant

Name of extract/standard	R_f values
<i>A. digitata</i> (Fruit)	0.03, 0.06, 0.11, 0.61
<i>A. squamosa</i> (Leaf)	0.03, 0.08, 0.83, 0.93
<i>B. variegata</i> (Bark)	0.61
<i>C. amada</i> (Rhizome)	0.66, 0.70, 0.73
<i>C. tora</i> (Leaf)	0.05, 0.08, 0.11, 0.61, 0.7, 0.81, 0.9, 0.95
<i>D. indica</i> (Seed)	0.7, 0.73, 0.85
<i>P. pterocarpum</i> (Leaf)	0.08, 0.13, 0.52*, 0.65, 0.67, 0.71, 0.76, 0.91
<i>P. pterocarpum</i> (Bark)	0.02, 0.07, 0.15, 0.52*, 0.68, 0.76, 0.92
<i>P. granatum</i> (Fruit epicarp)	0.07, 0.26, 0.32, 0.41, 0.52*, 0.7, 0.73, 0.8, 0.85
<i>S. cumini</i> (Leaf)	0.07, 0.11, 0.21, 0.28, 0.36, 0.52*, 0.62, 0.7, 0.72, 0.8, 0.86, 0.91
<i>S. xanthocarpum</i> (Aerial part)	0.03, 0.06, 0.38, 0.52*, 0.71, 0.78, 0.83
Gallic acid (Standard)	0.52

* shows presence of gallic acid in plant



A B C D E F

Figure 3: Detection of saponins by TLC in plants A and B = *A. digitata* fruit 4 μ l and 8 μ l C and D = *A. squamosa* leaves 4 μ l and 8 μ l E and F = *S. cumini* leaves 4 μ l and 8 μ l.

Table 11: R_f values of flavonoids detected in plant

Name of extract/plant	R_f values ^a	R_f values ^b
<i>A. digitata</i> (Fruit)	0.08	0.4, 0.57, 0.63, 0.68
<i>A. squamosa</i> (Leaf)	0.8, 0.88	0.2, 0.32, 0.53*
<i>B. variegata</i> (Bark)	0.47	0.46, 0.88
<i>C. amada</i> (Rhizome)	0.5	0.73, 0.83
<i>C. tora</i> (Leaf)	0.1, 0.23*, 0.3, 0.45, 0.48	0.21, 0.35, 0.46, 0.53*, 0.72, 0.82
<i>D. indica</i> (Seed)	0.57, 0.71, 0.86, 0.91	0.45
<i>P. pterocarpum</i> (Leaf)	0.27, 0.6, 0.72, 0.76, 0.83, 0.91, 0.93	0.43, 0.52, 0.58, 0.65, 0.76
<i>P. pterocarpum</i> (Bark)	0.26, 0.33, 0.47, 0.66, 0.86, 0.9	0.41, 0.48, 0.56, 0.6, 0.77
<i>P. granatum</i> (Fruit epicarp)	0.25, 0.27, 0.38, 0.65, 0.67, 0.72, 0.85	0.47, 0.8
<i>S. cumini</i> (Leaf)	0.41, 0.47, 0.53, 0.6, 0.7, 0.76, 0.78, 0.81, 0.86, 0.9	0.63, 0.78, 0.86
<i>S. xanthocarpum</i> (Aerial part)	0.27, 0.41, 0.63, 0.71, 0.82	0.45, 0.48, 0.58, 0.72, 0.81
Quercetin	0.23	-
Rutin	-	0.53

a- R_f values for solvent system n-hexane: ethyl acetate: glacial acetic acid (31:14:5)

b- Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26)

* shows matching of presence of quercetin or rutin in plant

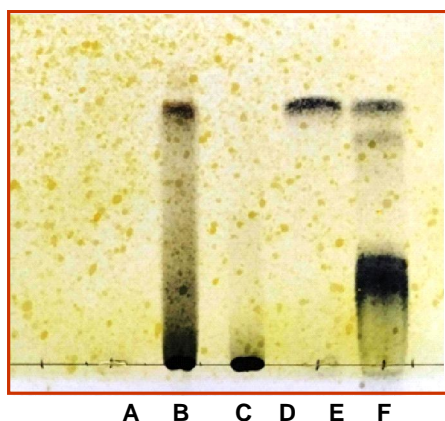


Figure 4: Detection of tannins by TLC in plants; A = *B. variegata* bark; B = *P. pterocarpum* bark; C = *P. granatum* fruit epicarp; D = gallic acid; E = tannic acid; F = mixture of tannin acid and gallic acid (increasing R_f).



Figure 5: Detection of sterol by TLC in plants; A = *B. variegata* bark; B = *S. xanthocarpum* aerial part.

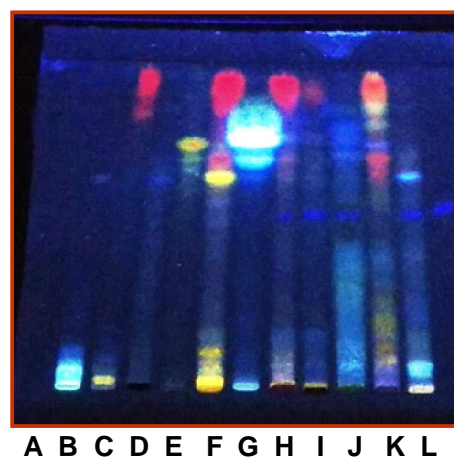
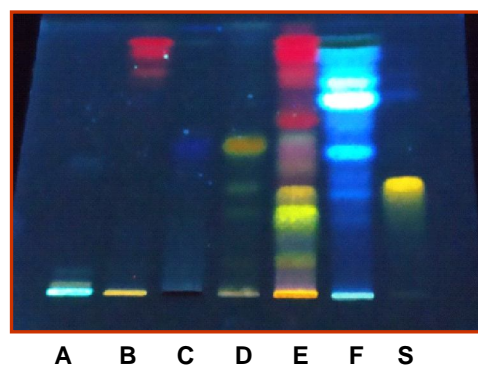
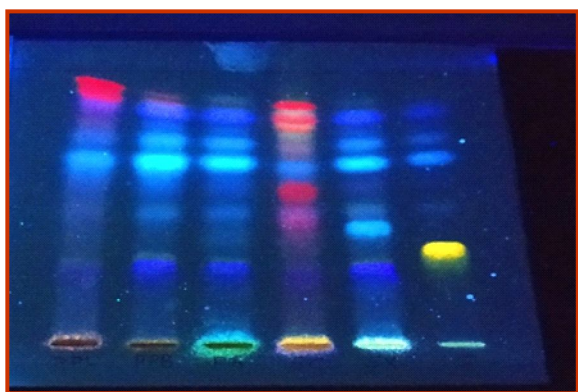


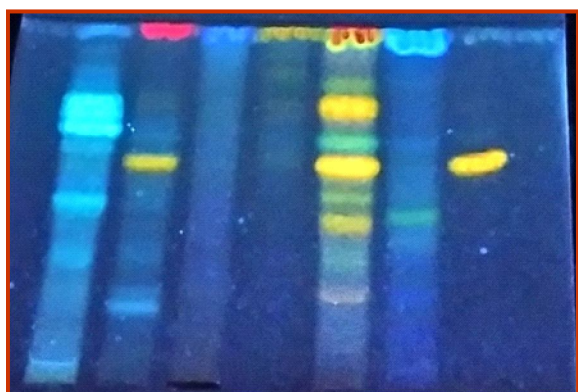
Figure 6: Detection of phenolic compounds by TLC in plants; A = *A. digitata* fruit; B = *A. squamosa* leaf; C = *B. variegata* bark; D = *C. amada* rhizome; E = *C. tora* leaf; F = *D. indica* fruit; G = *P. pterocarpum* leaf; H = *P. pterocarpum* bark; I = *P. granatum* fruit epicarp; J = *S. cumini* leaf; K = *S. xanthocarpum* aerial part; L = galic acid



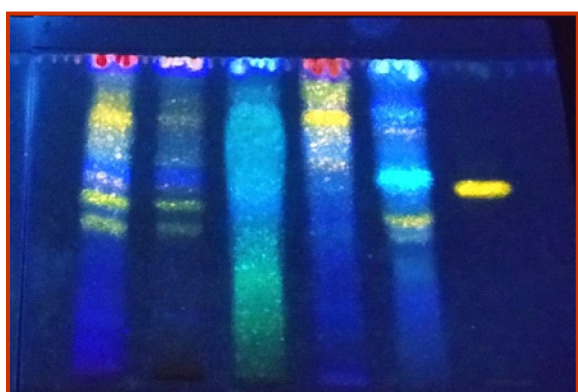


G H I J K S

Figure 7: Detection of quercetin by TLC in plants; A = *A. digitata* fruit; B = *A. squamosa* leaf; C = *B. variegata* bark; D = *C. amada* rhizome; E = *C. tora* leaf; F = *D. indica* fruit; G = *P. pterocarpum* leaf; H = *P. pterocarpum* bark; I = *P. granatum* fruit epicarp; J = *S. cumini* leaf; K = *S. xanthocarpum* aerial part; S = Quercetin.



A B C D E F S



G H I J K S

Figure 8: Detection of rutin by TLC in plants; A = *A. digitata* fruit; B = *A. squamosa* leaf; C = *B. variegata* bark; D = *C. amada* rhizome; E = *C. tora* leaf; F = *D. indica* fruit; G = *P. pterocarpum* leaf; H = *P. pterocarpum* bark; I = *P. granatum* fruit epicarp; J = *S. cumini* leaf; K = *S. xanthocarpum* aerial part; S = Rutin.

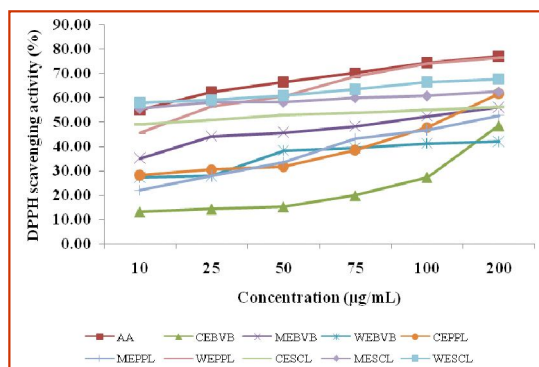


Figure 9: DPPH scavenging activity of different extracts of *B. variegata* bark, *P. pterocarpum* leaves, *S. cumini* leaves and ascorbic acid at various concentrations. (AA - Ascorbic acid; CEBVB - chloroform extract of *B. variegata* bark; MEBVB - methanol extract of *B. variegata* bark; WEBVB - Water extract of *B. variegata* bark; CEPPL - chloroform extract of *P. pterocarpum* leaves; MEPL - methanol extract of *P. pterocarpum* leaves; WEPL - water extract of *P. pterocarpum* leaves; CESCL - chloroform extract of *S. cumini* leaves; MESCL - methanol extract of *S. cumini* leaves; WESCL - water extract of *S. cumini* leaves).

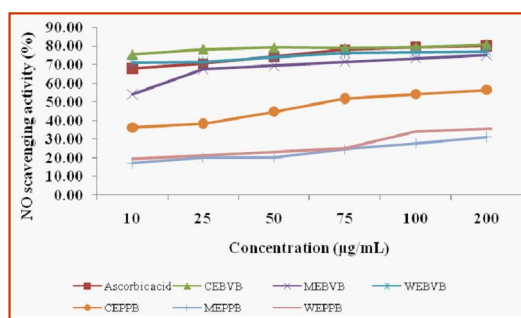


Figure 10: Nitric oxide scavenging activity of different extracts of *B. variegata* bark, *P. pterocarpum* bark and ascorbic acid at various concentrations (CEBVB - chloroform extract of *B. variegata* bark; MEBVB - methanol extract of *B. variegata* bark; WEBVB - water extract of *B. variegata* bark; CEPFB - chloroform extract of *P. pterocarpum* bark; MEPPB - methanol extract of *P. pterocarpum* bark; WEPPB - water extract of *P. pterocarpum* bark).

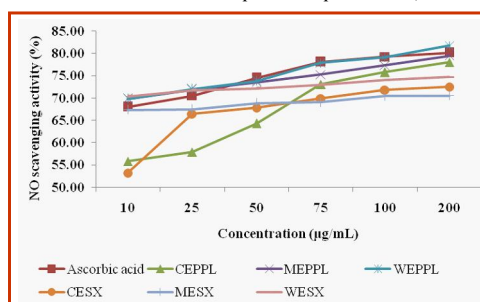


Figure 11: Nitric oxide scavenging activity of different extracts of *P. pterocarpum* leaves, *S. xanthocarpum* aerial part and ascorbic acid at various concentrations (CEPPL - chloroform extract of *P. pterocarpum* leaves; MEPL - methanol extract of *P. pterocarpum* leaves; WEPPL - water extract of *P. pterocarpum* leaves; CESX - chloroform extract of *S. xanthocarpum* aerial part; MESX - methanol extract of *S. xanthocarpum* aerial part; WESX - water extract of *S. xanthocarpum* aerial part).

4. Discussion

Phytochemical screening was performed as per standard tests. All these tests are qualitative which give an indication about the presence of particular phytochemical in the plant/extract based on the observation of either colour or secondary reactions. In the present study, total phenolic content (mg GAE per gram) in all plant extracts have also been determined. The Folin-Ciocalteu (FC) method is widely used to assay the total phenolic content (Folin and Ciocalteu, 1927). Total phenolic content can give an indirect idea about the antioxidant capacity of the plant extract (Oliveira *et al.*, 2012). Water extract of *P. pterocarpum* bark showed the highest phenolic content 72.73 mg/g GAE. This is due to the presence of numerous phytochemicals like lupeol, β -sitosterol, leucocyanidin and bergenin, *etc.* (Jash *et al.*, 2014). Few plants showed a lower level of total phenolic content which might be due to the presence of the fatty or organic compound. Apart from this, *A. squamosa*, *S. xanthocarum* and *S. cumini* exhibited the presence of alkaloid compound in different extracts.

Since one compound may have a different mechanism of antioxidant effect, so only one activity is not sufficient to describe the antioxidant activity of any test compound (Huang *et al.*, 2005). DPPH scavenging action is the most widely used method for determination of antioxidant activity. DPPH dissolves in methanol or ethanol and has absorbance maxima at 515 or 519 nm. When this free radical is scavenged, the colour of DPPH becomes lighter (Li *et al.*, 2009).

Out of ten plants, water extract of *P. pterocarpum* leaves showed inhibition of DPPH at $76.37 \pm 0.42\%$ which was comparable ($p > 0.05$) with ascorbic acid ($77.08 \pm 0.31\%$) at 200 $\mu\text{g/ml}$. Devi and Battu (2018) reported that *P. pterocarpum* leaves are the rich source of flavonoids like naringenin, ophioglonin, kaempferol isorhamnetin, luteolin, chrysoeriol, quercetin, *etc.* Chew *et al.* (2011) also reported *P. pterocarpum* as a potential antioxidant plant from the Leguminosae family. *S. cumini* is one of the most widely used plants in many diseases in traditional medicine, particularly in diabetes. The plant has been reported with a number of phytochemicals like anthocyanins, glucoside, ellagic acid, isoquercetin, kaempferol and myricetin (Ayyanar and Subhashbabu, 2012). All these phytochemicals are strong antioxidants and might be responsible for the strong DPPH inhibition in methanol and water extract. Methanol and water extracts of *S. cumini* leaves produced inhibition up to $62.46 \pm 0.40\%$ and $67.49 \pm 0.51\%$, respectively. However, the activity of *S. cumini* leaves was significantly ($p < 0.05$) lower as compared to ascorbic acid at the highest concentration. The result from DPPH inhibition in the present study indicates that extracts of few possess the ability to donate protons to free radicals and, thus can be used as a source of antioxidants as the antioxidant effect of extracts on DPPH was might be due to their ability to donate hydrogen to free radicals.

The damage caused by nitric oxide and super oxide radicals is multiplied as they react to produce reactive peroxy nitrile (ONOO⁻), which leads to serious toxic reactions with biomolecules. Generally, reactive free radicals like nitric oxides produced at a cellular level and add more reactive species (*e.g.*, peroxy nitrite), which are decomposed to hydroxyl radicals. In the present study, all the extracts of *B. variegata* bark also exhibited relatively high nitric oxide inhibition at 200 $\mu\text{g/ml}$ which was at par ($p > 0.05$) with per

cent inhibition by ascorbic acid. Singh *et al.* (2016) reported the presence of a number of phytochemicals like kaempferol, lupeol and beta-sitosterol, isoquercitroside, rutoside myricetol glycoside and kaempferol glycosides, bauhinione, *etc.*, in *B. variegata* stem bark which might cause the strong nitric oxide scavenging activity in the present study. Chloroform, methanol and water extract of *P. pterocarpum* leaves also exhibited inhibition of nitric oxide, $77.99 \pm 0.34\%$, $79.49 \pm 0.23\%$, $81.77 \pm 0.58\%$, respectively which were also at par ($p > 0.05$) with per cent inhibition by ascorbic acid. Numerous phytochemicals have been reported from the *P. pterocarpum* leaves which possess strong antioxidant actions like phenoxchromone derivatives and naringenin (Cavia-Saiz *et al.*, 2010), ophioglonin, luteolin (Majewska *et al.*, 2011), quercetin (Zhang *et al.*, 2011), quercitrin, chrysoeriol (Mishra *et al.*, 2003), *etc.* Similarly, all the extracts of *S. xanthocarum* showed comparable inhibition ($p > 0.05$) as shown by ascorbic acid at 200 $\mu\text{g/ml}$. A special type of glycol-alkaloid name solasodine has been found in all parts of the plant which might be responsible for its antioxidant action (Koduru *et al.*, 2007; Parmar *et al.*, 2010).

The mechanism and working of both the assay; DPPH (1, 1-diphenyl-2-picrylhydrazyl) and nitric oxide scavenging action are different. DPPH is a stable free radical which can be reduced to non-radical upon addition of one hydrogen molecule from the oxidized molecule (phytochemicals). Upon reduction the DPPH, produces stable yellow colour from dark violet colour and the intensity of colour is a measurement of ability of compound to scavenge the DPPH. That is why, the assay is sometimes referred to as free radical scavenging activity. The mechanism of nitric oxide assay is reverse of DPPH assay. In this case, nitric oxide is oxidized itself to become stable nitrate (NO₃⁻) and nitrite (NO₂⁻) under aerobic condition. The amount of stable ion can be detected quantitatively with the help of Griess reagent which produces pink colour by diazotization reaction (Alam *et al.*, 2013).

In the present study, upon thin layer chromatographic analysis, quercetin, rutin, gallic acid, other flavonoid and phenolic compounds have been identified in a few plants. Flavonoids like quercetin and rutin are used for the prevention and treatment of various ailments due to oxidative stress. Flavonoid acts as an antioxidant in biological systems and scavenges the free radicals, leads to strengthening the antioxidant defence system in the body. Among polyphenolic compounds, several factors could be responsible for increasing radical scavenging effectiveness. Those factors are including ortho-dihydroxy structure in the B ring, 2,3-double bond in conjugation with a 4-oxo function in the C-ring, hydroxy groups in positions 3 and 5 in the A ring, or the angle between the rings in the compound structure (Dugas *et al.*, 2000; Lopez-Vele *et al.*, 2003; Pawlak *et al.*, 2010). Presence of quercetin, rutin, gallic acid, other flavonoid and phenolic compounds in extracts of few plants might be responsible for their *in vitro* antioxidant activity.

5. Conclusion

Amongst, all plants selected and used to evaluate *in vitro* antioxidant activity in the present study, various extracts of *B. variegata*, *P. pterocarpum* and *S. cumini* showed remarkable DPPH scavenging activity. Various extracts of *B. variegata* and *S. Xanthocarum* also showed nitric oxide scavenging activity comparable to ascorbic acid. The plants like *B. variegata*, *P. pterocarpum* and *S. cumini* have a higher level of phenolic or flavonoid compounds which are

responsible for their antioxidant potential. The findings of the present study suggested that *B. variegata* bark, *P. pterocarpum* leaves, and *S. cumini* leaves may be a good source of natural antioxidant agents with great therapeutic importance for prevention of degenerative changes due to reactive oxygen species and associated oxidative stress condition.

Conflict of interest

The authors declare that no conflict of interest exists in the course of conducting this research. All authors had final decision regarding the manuscript and the decision to submit the findings for publication.

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