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Vol. 24(1) January-April, 2026

CONTENTS

Indian mustard: Phytoremediation tool for soil health management (Review) 1
M.L. DOTANIYA, C.K. DOTANIYA, KULDEEP KUMAR, JAKIR HUSSAIN, R.K. DOUTANIYA, H.M. MEENA and MANJU LATA

From profile to problems: A study on socio-economic status and constraints of rural women in dairy farming 14
ADITI PATHAK and ARPITA SHARMA KANDPAL

Diversification and intensification in rice–wheat cropping system for enhancing productivity under *Tarai* zone of Uttarakhand 20
PESALA SANTHA KUMAR, DINESH KUMAR SINGH, SUBHASH CHANDRA, ROHITASH SINGH and AJEET PRATAP SINGH

Sensor based nitrogen application impact on productivity, economics and nutrient uptake in maize 25
AMIT BHATNAGAR and AJAY KUMAR

Association of germination and seedling vigour attributes with green forage yield in oat (*Avena sativa* L.) genotypes through correlation and path coefficient analysis 31
DEVYANI RAMOLA, AKRITI BALLABH SANSKRITI JOSHI, MANISH MEHRA and BIRENDRA PRASAD

Genetic divergence and yield trait impact in French bean (*Phaseolus vulgaris* L.) 43
BHAWANA MAMGAIN, B.P. NAUTIYAL , S.C. PANT, VAISHALI THAPA, HEIGRUJAM RIYASANA and MAYANK

Arabic-gum based edible coatings preserving the post-harvest quality of Guava fruits 48
ESHA TEWARI and OMVEER SINGH

Efficacy of catechol and plant growth regulators on the vegetative growth and flowering of African Marigold (*Tagetes erecta* L. cv. Pusa Narangi Gainda) 59
SURYANSH SRIVASTAVA, AFSAR AHMAD, MD. RAMJAN and KHAN BILAL MUKHTAR AHMED

Host response of diverse pulse genotypes to root-knot nematode (*Meloidogyne incognita*) 67
SAROJ YADAV, EKTA RATHORE and ANIL KUMAR

Effect of egg size on egg quality traits, fertility, hatchability and day-old chick weight 75
ISLAM UDDIN SHEIKH, AZMAT ALAM KHAN, ARNAB JYOTI KALITA, SHEIKH ADIL HAMID, IRFAN AKRAM BABA, HENNA HAMADANI, BUSHRA ZAFFER and INSHA AFZAL

Pathomorphological studies on verminous pneumonia in a Gaddi goat 81
SWASTI SHARMA, SONALI MISHRA and R.D. PATIL

Pathological findings in congestive heart failure in dog: A case report 85
MUNISH BATRA and STUTI SINGH

Histopathological and haematological evaluation of the ameliorative effect of piperine on quinalphos-induced subacute toxicity in Swiss Albino Mice 89
PREETI SINGH, VINOD KUMAR, PREETI BAGRI and DEEPIKA LATHER

Phytochemical screening and antioxidant activities of locally available medicinal plants 99
PREETI BAGRI, VIJEYTA TIWARI, ARCHANA LOHIYA and VINOD KUMAR

Natural Farming Inputs (NFIs) as sustainable alternatives for enhancing the growth and development of Chickpea (*Cicer arietinum* L.) 108
SHAIFALI and A.K. SHARMA

Integrating Bayesian Calibration, Hierarchical Spatial Regression, and Structural–Temporal Models to Explain and Predict Wheat Yield in the Tarai–Bhabar Belt of Uttarakhand, India 114
NEHA BHATT and VINOD KUMAR

Phytochemical characterization and immuno-antioxidant potential of *Moringa oleifera* leaf extract in chicken lymphocytes 128
ANAMITRA RUJ, SONU AMBWANI and LATA PALIWAL

Biochemical changes associated with triclosan exposure in the freshwater fish *Cyprinus carpio* 140
MADHU SHARMA, YASHPAL, RIJUL RANA, DEEKSHA, DEEPIKA THAKUR and TARANG KUMAR SHAH

Assessment of awareness among respondents towards Pradhan Mantri Jan Dhan Yojana: A study in Haryana 148
MEGHA GOYAL, SUMAN GHALAWAT, MINAKSHI, ATUL DHINGRA and MINI

Youth perception and constraints in agri-entrepreneurship: A factor analytic study 156
JYOTI, SUMAN GHALAWAT, MEGHA GOYAL, NEELAM KAUSHAL, SUNIL KUMAR and VIKRANT HOODA

Design of Object Detection Model Using Deep Learning for Autonomous Driving in Adverse Weather Conditions: A GAN-Based Restoration and Depth-Aware Fusion Framework 165
MUMUKSHU BHATT, SUBODH PRASAD, RAJESH SINGH, ASHOK KUMAR and POOJA TAMTA

A novel mechanical purification approach for *Grewia optiva* Fibre-Reinforced epoxy composites: Effect of fibre geometry on structural performance 172
DEEPA SINGH , NEERAJ BISHT, TARUN KUMAR SAXENA and KAMAL RAWAT

Evaluating Long-Term rainfall and temperature trends across the Shipra River basin 184
POONAM SINGH, MOHAN LAL, DHEERAJ KUMAR, P. K. SINGH and GOVIND VERMA

Rainfall-runoff modelling using soft computing techniques for various watersheds of Madhya Pradesh, India 196
SEEMA KUSHWAHA and PRAVENDRA KUMAR

Prevalence and associated risk factors of functional constipation among the selected adult population of Pantnagar, U.S. Nagar, Uttarakhand, India 204
JYOTI SINGH, NEETU DOBHAL, ARCHANA KUSHWAHA, N.C SHAHI and SABBU SANGEETA

Tracking nutritional transitions: A comparative study of child malnutrition (0–5 years) trends across districts of Madhya Pradesh 212
SHALINI CHAKRABORTY

Determinants influencing tourist selection of rural tourism destinations using Garrett Ranking Technique: Evidence from Supi village, Uttarakhand 221
NEHA PANDEY and KAMAL UPRETI

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Phytochemical screening and antioxidant activities of locally available medicinal plants

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ABSTRACT: The objective of present study was to analyse phytochemical constituents and antioxidant properties of aqua-methanolic extracts obtained from the leaves of *Mangifera indica, Azadirachta indica*, *Ocimum sanctum* and *Psidium guajava.* In this investigation, phytochemical analysis for the important chemical constituents and antioxidant activity of extract was carried out. The phytochemicals such as phenols, flavonoids, tannin and non- tannin were determined quantitatively. The antioxidant property of the extracts was evaluated using *in vitro* assays *i.e.,* 2, 2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS. +) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). The total phenolic, total flavonoids and tannin contents were maximum in extract of leaves of *Mangifera indica.* The IC_{50} values was determined and revealed that *Psidium guajava* extract was better scavengers of ABTS and DPPH radicals.

Key words: ABTS, Antioxidant**,** DPPH, Total phenol

Despite the tremendous advancements in modern medical science, traditional medicine continues to be employed in developing nations to treat the bulk of illnesses (Bouguellid *et al*., 2022; Ferreira *et al*., 2023; Bagri and Kumar, 2024). Nearly 80% of the world's population relies on traditional medicines for primary health care, mast of which involve the use of plant extracts. In India almost 95% of the prescriptions were used in Unani, Ayurveda, Homeopathy and Siddha (Ramamurthy and Sathiyadevi, 2017). The importance of medicinal plants has enhanced together with the number of investigations into their biological effects on human beings and animals (Chandel *et al.,* 2011). Due to potential health benefits, plant-derived antioxidants, particularly, the phenolics have gained considerable significance. Epidemiological studies have shown that consumption of plant foods that contain antioxidants is important to health as it down-regulates several degenerative processes and also lower the occurrence of cancer and cardio-vascular diseases (Latif and Nawaz, 2025).

Medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry (Damani and Topi, 2022). The earliest mention of medicinal use of plants in Hindu culture is found in "Rigveda" which is said to have written between 4500-1600 BC (Sharma *et al.,* 2023). It is Ayurveda, the foundation of medicinal science of Hindu culture in its eight division deals with specific properties of drugs and various aspects of science of life and art of healing. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grown in different parts of the country.

Reactive oxygen species are essentially produced as the intermediate metabolites in a number of physiological processes like energy production in mitochondria, phagocytosis, cell growth regulation, intracellular signalling and detoxification of xenobiotics in the human body. Exposure to UV irradiations, chemical pollutants, organic solvents, tobacco smoke and pesticides are some of the environmental factors contributing to reactive oxygen species (ROS) over production. Stress generated due to current life style further adds to the overproduction of free radicals and causing tissue damage leading to deteriorating health conditions. High and chronic stress is related to a large number of pathological conditions like atherosclerosis, arthritis, ischemia, cancer and injuries to many

tissues including central nervous system (Myszko *et al.,* 2025). Human health is the primary concern as it influences the efficiency and indirectly affects the economic status of individual, family, society and nation. Use of antioxidants to combat increasing oxidative stress under normal and pathological conditions is exponentially rising. Application of antioxidants finds a new dimension in food technology to improve the shelf life of eatables. Synthetic antioxidant is reported to have various side effects including carcinogenicity and potential risks to human health, hence research on natural antioxidants and their applications is the need of the hour.

Polyphenols are the secondary metabolites of plants, synthesized under environmental stress. The phytochemicals and secondary metabolites of plants contribute to their medicinal values. Polyphenols absorb, quench and neutralize free radicals, act as reducing agents, metal chelators and can efficiently protect biological systems from degeneration under high oxidative stress (Rudrapal *et al.,* 2022). Antioxidants from plant sources, especially polyphenolic compounds effectively impede rancidity due to lipid oxidation in foods as well as development of oxidative stress related diseases (Parveen *et al.*, 2025). Industrial use of phenolics to improve food quality, shelf life and nutritional value (Mihaylova, 2024) is attracting researchers to find natural, safe components with better efficiency from plants. Flavonoids, a class of polyphenolics with free radical scavenging properties are known to inhibit hydrolytic and oxidative enzymes, reduce blood glucose and lipids, exhibit anti-inflammatory effect and enhance immunity in human beings (Zahra *et al.*, 2024). Therefore, flavonoids have attracted attention as a possible reply to various health issues especially related to oxidative stress.

Holy basil (*Ocimum sanctum* L), commonly grown in Indian subcontinent for its religious sanctity, is mentioned in the Charaka Samhita for its diverse medicinal properties including anti-hyperlipedemic, hypoglycemic, anxiolytic, hypotensive, anti-inflammatory and antimicrobial (Sahu, 2023). It is also considered to be an adaptogen being helpful for adapting to stress. In the traditional (ayurvedic and unani) medical systems, the extracts of holy basil leaves are used for common colds, inflammation and headaches (Lopresti *et al*., 2022).

Azadirachta indica Juss. (Neem, Family; Meliaceae) is a fast-growing evergreen popular tree found commonly in India, Africa and America. It has been used in Ayurvedic medicine for more than 4000 years due to its medicinal properties. The people of India have long revered the neem tree; for centuries, millions have cleaned their teeth with neem twigs, smeared skin disorders with neem leaf juice, taken neem tea as a tonic and placed neem leaves in their beds, books, grain bins, cupboard and closets to keep away troublesome bugs. The number of benefits of neem is listed in ancient documents like *Charak Samhita* and *Susruta Samhita*. It is commonly called 'Indian Lilac' or 'Margosa' (Tufail *et al.,* 2025).

Mango (*Mangifera indica* L.) is one of the most important tropical fruits marketed in the world. Mango is considered as a good source of dietary antioxidants such as phenolic compounds, ascorbic acid, and carotenoids. Mangiferin, an important component of mangoes, has antioxidant qualities and a redox-active aromatic system, making it a possible treatment for metabolic diseases (Moorthi, 2025).

Psidium guajava or guava is a member of family Myrtaceae with 133 genera and more than 3,800 species. Different parts of plant contain variety of phytochemicals with various pharmacological properties (Joshi *et al.*, 2023). Leaves of the plant are applied on wounds, ulcers, chewed to reduce toothache and are used in folk medicines since ancient times (Patil *et al.*, 2025; Ghuge and Khandre, 2024). Leaves of the plant contain resins, tannins, terpenoids, phenols, flavonoids, resins and show hepatoprotective, antimicrobial, antidiarrheal, biocidal, antimicrobial on periodontal pathogens, antimalarial and antioxidant activity and cure gastric ulcer disorder (Jassal and Kaushal, 2019).

So, the present study is designed to study phytochemical constituent and evaluating the antioxidant activity of aqua-methanolic extract

obtained from the leaves of *Mangifera indica, Azadirachta indica*, *Ocimum sanctum* and *Psidium guajava*.

MATERIALS AND METHODS

Collection, identification and authentication of various locally available medicinal plants

The leaves of selected medicinal plants i.e., *Mangifera indica, Azadirachta indica*, *Ocimum sanctum* and *Psidium guajava* were collected from Medicinal, Aromatic and Under-utilized plant section of Department of Genetics and Plant Breeding, College of Agriculture, CCS HAU vide letter no. VPTX/2019/1998 dated 19.10.2019. Identification and authentication of different selected plant were carried out by consultant botanist. Department of Botany, CCSHAU, Hisar vide letter no. VPTX/2019/1999 dated 24.10.2019.

Preparation of different extracts

The leaves of selected medicinal plants i.e., *Mangifera indica, Azadirachta indica*, *Ocimum sanctum* and *Psidium guajava* were shade dried at room temperature. The leaves were grinded, powdered and stored at room temperature. The powder of these were soaked in solvent 50 % methanol (aqua-methanol; AM) for 24 hours at room temperature with intermittent shaking and followed by heating at 38ºC with intermittent shaking. The mixtures were then filtered through filter paper. The filtrates were dried in vacuum rotary evaporator at 40ºC followed by further drying on water bath for obtaining the different extracts.

$$\text{Total extract yield, Y (\%)} = \frac{\text{Total mass of extract}}{\text{Total mass of sample}} \times 100$$

(Kamarudin *et al.*, 2016)

Preliminary Phytochemical Screening

The extracts of different plants were subjected to different chemical tests for the detection of different phytoconstituents using standard procedures

a) **Alkaloids Test**: Mayer's reagent test- 10 mg of extract was dissolved in 1N HCL solution. Then 1 ml of extract was taken and 5-6 drops of Mayer's reagent (1.5 g Mercuric chloride and 5 g potassium iodide in 100 ml water) was added. Formation of white precipitate indicated presence of alkaloids.

b) **Phenols:** Ferric chloride test- 2 ml plant extract was taken in water and warmed at 45 -50 ºC. Then add 2 ml of 0.3% ferric chloride was added. Formation of green or blue color indicates presence of phenols.

c) **Glycosides:** Fehling's Test- Fehling's solution A and B was diluted with distilled water and boiled for 1 min. To this clear blue solution, 8 drops of plant extract was added. After that it was mixed with 1 ml of Fehling's solution and boiled in water bath for 5 min. The formation of brick red precipitation indicates presence of glycosides.

d) **Tannin:** Ferric chloride test- 20 mg extract solution in methanol was taken and heated for 2- 4 min, then filter through filter paper. The filtrate was used to conduct the ferric chloride test. A few drops of 0.1% $FeCl_3$ was added in the above filtrate and observed for brownish green black or blue-black coloration.

e) **Resin:** A small amount (20 mg) of extract was dissolved in alcohol and a few drops (5-6) of distilled water were added. The appearance of turbidity was indicative of resin.

f) **Proteins:** Xanthoprotein test- 1 ml of extract was taken in 2ml of water to this. 0.5 ml of concentrated nitric acid was added. The appearance of white or yellowish precipitate indicated the presence of protein.

Quantitative Determination of Phytochemical Constituents

a) **Determination of total phenolic content:** Total phenolic content in the samples was estimated by Folin-Ciocalteu (FC) assay (Kumar *et al.,* 2019) on a microplate reader 96 well plate using gallic acid as the standard. A 50 µl of the diluted extracts (1mg/ml) or gallic acid dilutions (2.5–100 µg/ml) and 50 µl of diluted FCR (1:5) were placed in each well of microtiter plate. After a few seconds, 100 µl of sodium hydroxide (0.35 M) was added and the mixture was shaken at medium-continuous speed for about 1 min at room temperature. The absorbance

of the blue-colored reaction mixture was measured at λ_{max} 765 nm against blank after 3 min using the microplate reader at room temperature. All the extracts were screened in triplicate. The phenolic content equivalent to gallic acid was estimated. The results were expressed as gallic acid equivalents (mg GAE/g of extract)

b) **Determination of total flavonoid content:** Total flavonoids content (TFC) was assessed by an assay based on aluminium chloride ($AlCl_3$) complex (Kumar *et al.,* 2019) in all the plant extracts. The test sample of methanolic solution (100 µl, 1 mg/ml) was mixed with 2% w/v $AlCl_3$ (100 µl) into each well of microtiter plate. After incubating the samples for 15 min at room temperature, the absorbance was recorded at λ_{max} 435 nm using the microtiter plate reader. All the samples were tested in triplicates. Relative activities were calculated from the calibration curve of quercetin standard solutions (ranging from 1 to 100 µg/ml) working in the same experimental conditions. The total flavonoid content was expressed as quercetin equivalent in milligrams per gram of the extract.

c) **Tannin and non-tannin contents:** Tannin content in each sample was determined using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannins as described by Makkar *et al.* (1993). Briefly, 1 ml of extract dissolved in methanol (1 mg/ml), in which the total phenolics were determined, was mixed with 100 mg PVPP, vortexed, left for 15 min at 4°C and then centrifuged for 10 min at 3,000 rpm. In the clear supernatant the non-tannin phenolics were determined the same way as the total phenolics. Tannin content was calculated as a difference between total and non-tannin phenolic content.

Tannin = Total Phenolic content – non-tannin phenolic content

***In vitro* antioxidant activity**

Antioxidant assays- The extracts of herbal plants were screened *in vitro* for their antioxidant activities by following assay

a) **2, 2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\cdot +}$) radical scavenging:** The total antioxidant activity of different extracts was determined according to the standard method (Lee *et al.,* 2015) based on ABTS radical cations ($ABTS^{\cdot +}$) scavenging assay. A 2mM ABTS was taken in volumetric flask and 100 ml double distilled water was added. $ABTS^{\cdot +}$ were produced by mixing the stock solution with 400 µl of 70 mM potassium persulfate. To ensure complete oxidation of ABTS, the mixture was held at room temperature in the dark for 6 to 12 hr prior to analysis. The resulting $ABTS^{\cdot +}$ solution was diluted with phosphate buffer (about four-fold) to give an absorbance reading of 0.750 at 734 nm. To determine the scavenging activity, 100 µL ABTS reagent was mixed with 100 µL of sample (10-400 µg/ml) in a 96-well microplate and was incubated at room temperature for 6 min. After incubation, the absorbance was measured 734 nm using an ELISA reader and 100% methanol was used as a control. The ABTS scavenging effect was measured using the following formula:

Radical scavenging (%) =

[(A) control - (A) sample (A) control] ×100.

The IC_{50} ABTS values (the concentration of sample required for inhibition of 50% of ABTS radicals) were obtained. The antioxidant activity was evaluated based on this IC_{50} value.

b) **2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging:** The 96-well plate method, established by Kumar *et al.* (2019) was used to evaluate the antioxidant activity of the extracts using ascorbic acid as the standard. Briefly, aliquots of the extracts (100 µl) prepared in methanol were allowed to react with an equal amount (100 µl) of DPPH reagent (0.2 mM) in each well. The same procedure was carried for ascorbic acid which was considered as the standard. Moreover, negative controls were prepared by mixing DPPH and methanol (100 µl each) in the wells. The mixture was stirred and kept in dark for 30 min. After incubation, a decrease in DPPH radical was analyzed by measuring absorption at λ_{max} 517 nm using microplate reader. The percentage of radical scavenging activity of samples was evaluated by comparing with control group. All the determinations were done in triplicate. % inhibition of DPPH was calculated by the following formula:

% Scavenging activity = [(A control –A sample or standard)/A control] × 100

RESULTS AND DISCUSSION

***Extraction Yield*:** The percentage recovery or extraction yield of aqua-methanolic extracts of *Mangifera indica, Azadirachta indica*, *Ocimum sanctum* and *Psidium guajava* leaves are presented in Table 1. The maximum percent recovery (33.6 %) was observed in the extracts of *Ocimum sanctum* among all four plants.

***Preliminary Phytochemical Screening*:** Different phytochemical like alkaloid, phenol, tannins, glycosides, resin and proteins are estimated qualitatively and results are presented in Table 2.

***Phytochemical analysis*:** The values of total phenols of different extracts were calculated from the standard curve of gallic acid and presented in Table 3. The total phenolic contents (GAE/gm of extract) were maximum in extract of leaves of *Mangifera indica* (173.54 ± 3.26 mg of GAE/gm of extract) and minimum in extract of *Azadirachta indica* (41.94± 1.76 mg of GAE/gm of extract), as compared with other extracts. The values of total flavonoids of different extracts were calculated from the standard curve of quercetin and presented in Table 3. The total flavonoids content was more in *Mangifera indica* (34.64 ± 1.11 mg quercetin Equiv./ g extract). However, the flavonoid content was minimum in *Psidium guajava* (13.76± 0.48 mg quercetin Equiv./ g extract).

The tannin and non-tannin contents present in the different types of extracts are given in Table 3. The proportion of tannin contents was more in the extracts of *Mangifera indica* whereas non tannin contents were more in *Psidium guajava*.

Table 1: Percentage extractability of different types of extracts (Mean ± SE)

S. No.	Type of extract	% extractability (Mean ± SE)
1	Mango (*Mangifera indica*)	19.83±4.56
2	Neem (*Azadirachta indica*)	21.01±4.24
3	Tulsi (*Ocimum sanctum*)	33.60±1.89
4	Guava (*Psidium guajava*)	20.08±3.81

***Antioxidant activity*:** The plant extracts showed the inhibition of the different *in-vitro* free radicals i.e., ABTS and DPPH in a concentration dependent manner up to some particular concentration. The inhibition of these ABTS and DPPH by some standard antioxidants (i.e. Trolox or BHT or Ascorbic acid) at different range of concentration. Finally, the IC_{50} values of the different extracts for the total antioxidant activity and free radical scavenging assay are presented in Table 4. The IC_{50} values was determined and revealed that *Psidium guajava* extract was better scavengers of ABTS and DPPH radicals.

Phytochemical screening of the plants revealed some differences in the constituents of the four plants tested. *Ocimum sanctum* tested positive for all the phytochemicals tested; *M. indica, Psidium guajava* and *Azadirachta indica* showed the absence of resins and protein whereas *Azadirachta indica* also showed the absence glycosides. Yield and chemical composition of extracts depend upon number of factors such as climatic conditions, geographical location and stage of plant (Atanasov *et al.,* 2021; Sun *et al.,* 2025). Our results had shown the presence of phenols, flavonoids, and tannins in these plant extracts which are among the most abundant and varied classes of natural compounds found in plant species. Flavonoids are found to be better antioxidants to have multiple biological activities including vasodilatory, anti-carcinogenic, anti-inflammatory, antibacterial, immune-stimulating, anti-allergic, antiviral and radioprotective effects (Stachelska *et al.,* 2025).

All the plants leave extract exhibited potent antioxidant activity. The presence of flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Sun and Shahrajabian, 2023). Nowadays, there is much interest in tracking the antioxidant activity of plant or food extracts to investigate possible

Table 2: Phytochemicals in aqua-methanolic extracts of *Mangifera indica, Azadirachta indica, Ocimum sanctum* and *Psidium guajava* leaves

Sr. No.	Phytochemical	*Mangifera indica*	*Azadirachta indica*	*Ocimum sanctum*	*Psidium guajava*
1.	Alkaloids	+	+	+	+
2.	Phenols	+	+	+	+
3.	Glycosides	+	-	+	+
4.	Tannin	+	+	+	+
5.	Resin	-	-	+	-
6.	Proteins	-	-	+	-

Table 3: Phytochemical parameters [total phenols, total flavonoids, tannin, non-tannin contents] of aqua-methanolic extracts of *Mangifera indica, Azadirachta indica, Ocimum sanctum* and *Psidium guajava* leaves (The values are expressed as Mean ± SE; n=3)

Type of extract	Parameters			
	Total Phenols (mg of GAE /gextract)	Total flavonoids (mg quercetinEqu./g extract)	Tannin contents (mg of GAE/gextract)	Non-Tannincontents (mg of GAE/gextract)
Mangifera indica	173.54± 3.26	34.64± 1.11	135.85 ± 1.49	37.70 ± 2.14
Azadirachta indica	41.94^{a}± 1.76	16.14^{a}± 0.04	20.64^{a} ± 1.38	21.30 ± 0.55
Ocimum sanctum	134.79^{b}± 2.73	19.0^{a}± 2.76	89.67^{ab} ± 11.28	45.12 ± 11.28
Psidium guajava	171.0^{abc}±5.45	13.76^{a}± 0.48	122.12^{bc} ± 6.09	48.88^{b} ± 0.66

Means bearing a, b and c superscripts differ significantly ($P \leq 0.05$) vs. *Mangifera indica*, *Azadirachta indica* and *Ocimum sanctum*, respectively

Table 4: IC_{50} values of different of aqua-methanolic extracts of *Mangifera indica, Azadirachta indica, Ocimum sanctum* and *Psidium guajava* leaves and some standards (Trolox, BHT and ascorbic acid) for ABTS and DPPH (The values are expressed as Mean ± SE; n=3)

Type of extractor standard	IC50 values	
	Parameters	
	Total antioxidantactivity (ABTS)(µg/ml)	Free radicalscavenging activity(DPPH) (µg/ml)
Mangifera indica	11.02±0.25	60.72±3.98
Azadirachta indica	41.34^{a}±2.55	448.68^{a}±6.39
Ocimum sanctum	16.20^{b}±0.96	46.52^{b}±0.54
Psidium guajava	8.64^{bc}±0.08	14.86^{abc}±0.67
Trolox	7.04^{bc}±0.54	-
BHT	6.25^{bc}±1.61	-
Ascorbic acid	-	5.98^{abc}±0.26

Means bearing a, b and c superscripts differ significantly ($P \leq 0.05$) vs. *Mangifera indica*, *Azadirachta indica* and *Ocimum sanctum*, respectively

medicinal properties. For the evaluation of antioxidant activity, one of the methods used was the DPPH (2,2-diphenyl-1-picrylhydraza) method, one of the most effective, simple, and reliable *in vitro* methods that has the ability to sequester free radicals (Souza *et al.*, 2020). The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517 nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. Martínez-Cabanas *et al.,* 2021 reinforce that due to the complexity of the chemicals present in crude extracts, it is necessary to evaluate the antioxidant capacity of the plant by at least two methods. In this sense, another method for evaluating antioxidant activity used was the ABTS method; it is being widely used among antioxidant analyses. Ayoola *et al.*, 2008 also reported that *Psidium guajava* showed maximum antioxidant activities as

compared to other plants. Our results have also shown the similar pattern, where *Psidium guajava* extract has shown the most potent antioxidant activity as compare to others plant used in this study and the values obtained are comparable to standard used in respective tests.

This study suggests that these plants possess antioxidant activities which can counteract the oxidative damage caused by UV irradiations, chemical pollutants, organic solvents, tobacco smoke and pesticides.

CONCLUSION

This study demonstrates in an unprecedented way that the crude extract of these plants leaves is rich in phenolic compounds and flavonoids and has the capacity to neutralize different sources of ROS. Thus, it is suggested that there is a synergism between the chemical composition of the extract, especially phenolic compounds, with the high antioxidant capacity demonstrated through different analysis techniques. Our results open the way for the possible development of natural antioxidants after further studies for the isolation of compounds and more specific *in vivo* investigations to elucidate the mechanisms of action of the extract at more complex cellular and organism levels, as well as its pharmacological evaluation.

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