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RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF ANTIBACTERIAL, ANTIOXIDANT AND PHOTOSYNTHETIC ACTIVITY OF *Azadirachta indica*, *Rosa indica* AND *Moringa oliefera* CULTIVARS

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ABSTRACT

In the present study, three plants viz. *Rosa indica* (petals), *Azadirachta indica* (leaves) and *Moringa oleifera* (leaves) were screened for potential antibacterial and antioxidant activity. To evaluate these activities two organic solvents i.e. acetone and ethanol were used to extract the antibacterial and antioxidant compounds from plant sample. Antibacterial activity was tested against two gram positive i.e. *Staphylococcus aureus*, *Bacillus pumilis* and two gram negative i.e. *Klebsiella pneumoniae*, *Escherichia coli* bacterial strains by agar well diffusion method. The ethanolic extracts of red rose petals were found to be most effective against all the pathogens tested. Antioxidant activity of all three plants was screened by measuring total phenolic content, total flavonoids and by free radical scavenging assay using DPPH. Significant differences in DPPH scavenging activity were reported between the species investigated, ranging from 74.72% to 83.40%. The total phenol content of the investigated species ranged from 74 to 96 mg CE/ g extract while flavonoid content ranged between 39 to 52 mg QE/g extract. In addition photosynthetic pigments (Chl A, Chl B, and carotene) were also determined for all three plants under study.

INTRODUCTION

Since many years, plants have been used as valuable sources of natural products for maintaining the human health. These plant products are being used not only in developing countries like India, rather also in developed countries even after there are huge amount of allopathic drugs available in the market. One reason of this maybe widespread use of drugs is leading to the development of resistance against them in the pathogen (Davis., *et al*., 2009) and also the side effects associated with them is urging people not to use them. Free radicals include hydroxyl, superoxide, nitric oxide, nitrogen dioxide, peroxyl, lipid peroxyl and hydrogen peroxide, which are generated by products of normal cellular metabolism. These free radicals are highly reactive and damaging to human body so their removal is essential. Many medicinal plants contain large amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein, and other Carotenoids which play important roles in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Djeridane, *et al*., 2006)

Rose Petals contains Anthocyanins and proanthocyanidins; tellimagrandin I and rugosin B; carotenoids; plant acids; and essential oils. High concentration of anthocyanins present in the rose petals indicates their importance because anthocyanins have powerful antioxidant, antibacterial and antinflammatory activity. Rose petal water can also be used as an eyewash and mouthwash (Akhmadieva *et al*., 1993). Neem has been extensively used in ayurveda, unani and homoeopathic medicine and has become a cynosure of modern medicine. Neem leaves mainly contains Cyclic trisulphide (Siddiqui *et al*., 1942) and cyclic tetrasulphide (Mitra, *et al*., 1963). Due to these cyclic compounds it contains antifungal and antibacterial activity. It is an established fact that polyphenolic compounds possess remarkable antioxidant activities which are present quite commonly in the plant family Meliaceae. *Moringa oleifera* is a highly valuable plant, distributed in many countries of the tropics and subtropics. It has a wide range of medicinal properties including antioxidant, antidiabetic, hepatoprotective (Ruckmani *et al*., 1998), antibacterial, antifungal activities (Nickon *et al*., 2003) and several others. Nickon *et al*., 2003 reported that moringa leaves contain the broad spectrum antibacterial properties comparing to other plant parts. The leaves of Moringa are highly nutritious, being a significant source of beta-carotene, vitamin C, protein, iron and potassium. (Johnson, 2005) The antioxidant activity and the total phenolic contents of *Moringa oleifera* leaves in two stages of maturity responsible for preventing the deleterious effects of oxidative stress were disclosed in a recent study (Sreelatha, *et al*., 2009). Due to presence of wide range of phytochemicals of medicinal importance present in different parts of these plants, the present study was focused to evaluate antibacterial and antioxidant properties of these three plants.

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MATERIALS AND METHODS

The present study was carried out at the laboratory of Molecular and Cellular Engineering Department, SHIATS, Allahabad. The work was designed to study the antioxidant, antibacterial and photosynthetic activity of three Indian medicinal plants viz. *Rosa indica*, *Azadirachta indica* (Neem) and *Moringa oleifera* (Drumstick).Table 1 below is showing the classification of investigated plants.

Table 1. Classification of investigated plant

Botanical Name	Family	Vernacular name	Plant Part Used
Rosa indica	Rosaceace	Rose	Red Flower Petals
Azadirachta indica	Meliaceae	Neem	Green Leaves
Moringa Oleifera	Moringaceae	Sehjan	Green Leaves

Collection of plant material

The fresh leaves and petals were collected from the Nursery of School of Forestry and Environment, SHIATS, Allahabad, U.P. (India)

Processing of plant material

The fresh leaves and petals were surface sterilized simply by washing under tap water and D.W. and dried in shed for 20 days. After drying, leaves and petals were grounded in a grinder mixer to a powdered form and stored for further use.

Preparation of plant extracts

For preparing the plant extracts, two organic solvents, viz. ethanol (70%) and acetone were used. The powdered leaves and petals (10 gm each) were extracted in a round bottom flask in 100 ml solvent (ethanol and acetone). Flasks were kept in dark room for 3 days at room temp wrapped with aluminium foil to avoid evaporation. After 3 days, mixtures were filtered through What man No.1 filter paper, and filtrate was kept in incubator at 37 ^{0}C till all solvents completely evaporated from mixtures. The remaining powdered extract was weighed and dissolved in double amount of DMSO (500mg/ml). It was further stored at 4 ^{0}C till use.

Antibacterial Screening

Tested microorganisms and preparation of their inoculums

The antibacterial activity of plant leaves and petals was tested against the four pathogenic bacteria viz. *Klebsiella pneumoniae, Escherichia coli, Bacillus pumilis, Staphylococcus aureus.* These four preisolated bacterial cultures were collected from Microbial Culture Collection Bank, Department of Microbiology and Fermentation Technology, SHIATS. These cultures were subcultured on NA slants and stored at 4 ^{0}C till use. For testing, the inoculums were prepared from the stock culture, which were sub cultured into nutrient broth (30ml) using a sterilized wire loop and incubated overnight in a rotary shaker at 37^{0}C. After overnight incubation, these inocula were further stored at 4^{0}C till use.

Antibiogram analysis (Disc Diffusion Assay)

The antibacterial activity of *Rosa indica* petals, *Azadirachta indica* and *Moringa oleifera* leaves was evaluated against four pathogenic bacterial strains by using agar well diffusion method under sterelized conditions. For this twelve NA plates were prepared for all plant extracts. 400µl inoculum of each selected bacterium was uniformly spreaded over agar plates with the help of sterilized glass spreader. After five minutes three wells, approximately 7mm in diameter were bored with the help of borer. The equal volume (50 µl) of antibiotic Tetracycline (100 mg/ml), ethanolic and acetone plant extract (200mg/ml) were poured into the wells. The plates were incubated at 37 ^{0}C for 24 hrs in incubator. The results were observed on the next day and the effectiveness was measured in terms of diameter of zone of inhibition (clear zone).

Antioxidant Activity Screening

Free radical Scavenging Activity Assay (DPPH method)

Free radical scavenging activity was evaluated using L- ascorbic acid as standard antioxidant. The radical scavenging activity was measured using the stable radical DPPH according to the method described by Chan *et al.,* 2007 with some modifications. Ethanolic plant extract concentration was maintained at 1mg/ml for each plant and different dilutions (0.2, 0.4, 0.6, 0.8, 1.0 mg/ml) were prepared. DPPH solutions were also prepared by dissolving 6mg of DPPH in 100 ml methanol. 1 ml of extract from each dilution was added into the test tube containing 2 ml of DPPH solution. The mixture was shaken vigorously and was left to stand in dark for 30 minutes. The absorbance was measured at 517 nm in a UV-VIS Spectrophotometer.

Calculation

% Scavenging activity = [(Absorbance of control - Absorbance of sample) / Absorbance of control] ×100

Determination of Total Phenolic Content (TPC)

It is reported that phenols are responsible for the variation in the antioxidant activity of the plant. (Cai *et al*., 2004). They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals. (Pokorny *et al.*, 2001, Pitchaon *et al.,* 2007).The extraction of total phenolics was performed using the Folin-ciocalteu assay, following the method of (Bray *et al.,* 1954) with some modifications. 1.0 gm dry powder of each plant sample was weighed and extracted in 10 times volume (10 ml) of 80% (v/v) ethanol. It was again centrifuged and supernatant was pooled out. The supernatant was evaporated to dryness and residue was dissolved in 10ml of distilled water. Catechol was used as standard. Different aliquotes of plant extract (0.5 and 1.0ml) were pipetted out in 2 test tubes and the volume was made up to 3 ml with D.W. 0.5 ml of FCR reagent was added to each test tube. After 30 minutes 2 ml of 20% (w/v) Na_2CO_3 solution was added to each test tube. Mixed the tubes thoroughly and kept in a boiling water bath for exactly one minute , cooled and absorbance was measured at 650 nm in a UV-VIS Spectrophotometer against a reagent blank. Standard curve was prepared using different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/ml) of catechol. Results are expressed in terms of mg CE/gram plant extract.

Determination of Total Flavonoid Content (TFC)

The TFC in ethanolic extract was determined according to Morena *et al*., 2000. A 0.5 ml plant ethanolic extract (1 mg/ml) was mixed with 0.1 ml of 10% (w/v) aluminium nitrate and 0.1 ml of potassium acetate (1 M) and 4.3 ml of 80% of ethanol was added to make a final volume of 5 ml. The mixture was vortexed and the solution was allowed to stand for 40 minutes for reaction at room temperature. The absorbance was measured spectrophotometrically at 415 nm. All determinations were performed in duplicate. Total flavonoid values are expressed in terms of mg QE per gram of plant extract. A standard curve was prepared using 0.02, 0.04, 0.06, 0.08, 0.10 mg/ml solutions of quercetin.

Determination of Photosynthetic Pigments (Chlorophyll A, B and Carotenoid)

Photosynthetic pigments (chlorophyll A, B and total carotenoids) in the leaves and petals of three plants, viz. *Azadirachta indica, Moringa oleifera* and *Rosa indica* were determined according to method given by Saric *et al*., 1976 with slight modifications. 200 mg of dried, powdered plant sample was taken in a round bottom conical flask, containing 10 ml (80% v/v) acetone. Sample was kept overnight in refrigerator at 4 ^{0}C and later on homogenized and centrifuged at 5000 rpm for 7 minutes. Supernatant was collected and washed several times until it became clear. The optical density of the final volume of supernatant was measured in a UV-VIS Spectrophotometer at 644 nm and 662 nm for chlorophyll and at 440 nm for carotenoids.

Calculation: for calculating the amount of photosynthetic pigments, following formula was used:

Chl A = 9.784 × E 662 – 0.99 × E 644 = X_1
Chl B = 21.426 × E644 – 4.65 × E662 = X_2
Carotene = 4.695 × E 440 – 0.268 ($X_1 + X_2$)
Where E = optical density at E(662,644,440)nm
The content was expressed in terms of mg/gm dry wt.
The content (mg/gm dry wt.) = x × Volume of acetone ÷ weight of sample (mg)

RESULTS AND DISCUSSION

Antibacterial Screening

Antibiogram analysis (Disc Diffusion Assay)

The results of antibiotic susceptibility test were recorded in terms of diameter of zone of inhibition (in mm) for extract of each cultivar in ethanol and acetone as solvents, against each bacterial strain used. The zone of inhibition was also recorded for the standard antibiotic (Tetracycline) against each strain. Data of antibacterial activity of various crude extracts prepared from dry leaves and petals are demonstrated in tabulated and graphical form.

Table 2-4 below, shows the results of antibacterial susceptibility assay observed for ethanolic and aetone extracts of all three cultivers against bacterial strains tested, using Tetracycline as a standard antibacterial agent.

Antioxidant Screening

Free radical scavenging activity (DPPH assay)

The antioxidant activity of medicinal plants is mainly related to their bioactive compounds, such as phenolics, flavonols, and flavenoids. In this study, the antioxidant capacity of ethanolic extracts of three Indian medicinal plants at different concentrations was systematically evaluated. The DPPH inhibition of different plant extracts is summarized in Figure 5 and Table 5.

Table 2. Antibacterial susceptibility assay of *Azadirachta indica* leaves extracts

Tested Bacteria	Zone of Inhibition (in mm.)		
	Acetone Extract	Ethanolic Extract	Tetracycline
E. coli	24.0 ± 1.0	20.0 ± 1.0	30.0 ± 2.0
K. pneumoniae	14.0 ± 1.0	14.0 ± 0.0	29.0 ± 1.0
S. aureus	16.0 ± 1.0	28.0 ± 1.0	31.0 ± 1.0
B. pumilis	25.0 ± 1.0	25.0 ± 1.0	28.0 ± 2.0
Well Diameter:7mm			

Table 3. Antibacterial susceptibility assay of *Rosa indica* petals extracts

Tested Bacteria	Zone of Inhibition (in mm.)		
	Acetone Extract	Ethanolic Extract	Tetracycline
E. coli	17.0 ± 1.0	22.0 ± 1.0	38.0 ± 2.0
K. pneumoniae	16.0 ± 1.0	20.0 ± 1.0	30.0 ± 2.0
S. aureus	14.0 ± 0.0	17.0 ± 0.0	34.0 ± 1.0
B. pumilis	21.0 ± 1.0	24.0 ± 1.0	35.0 ± 1.0
Well Diameter:7mm			

Table 4. Antibacterial susceptibility assay of *Moringa oleifera* leaves extracts

Tested Bacteria	Zone of Inhibition (in mm.)		
	Acetone Extract	Ethanolic Extract	Tetracycline
E. coli	No inhibition	No inhibition	33.0 ± 2.0
K. pneumoniae	10.0 ± 1.0	15.0 ± 1.0	30.0 ± 2.0
S. aureus	08.0 ± 1.0	11.0 ± 0.0	30.0 ± 1.0
B. pumilis	07.0 ± 1.0	10.0 ± 1.0	30.0 ± 1.0
Well Diameter:7mm			

Fig 1-4. below, are the plates showing the zone of inhibition (clear zone) of various plant extracts under study against tested bacterial strains.
Note- T=Tetracycline (Control), AE=Acetone Extract, EE=Ethanolic Extract

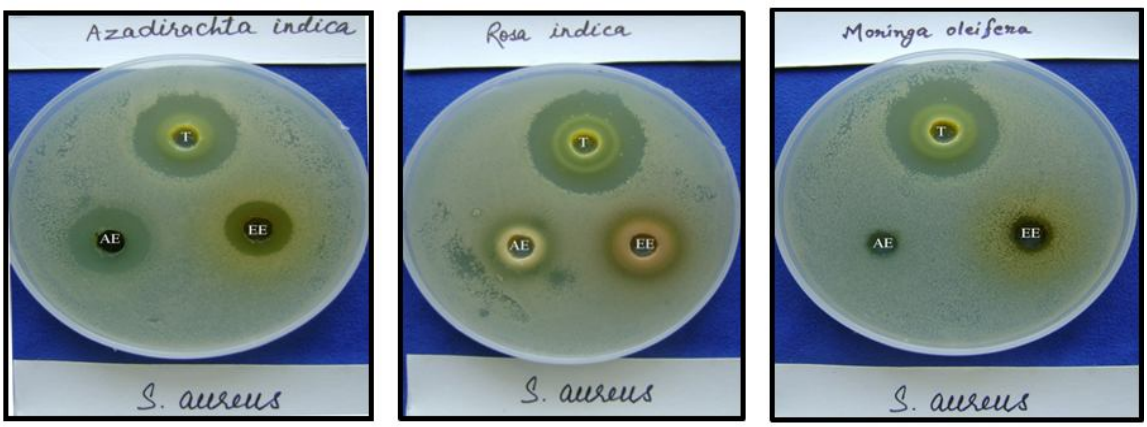

Figure 1. Antibiogram analysis of various plant extracts against *S.aureus*

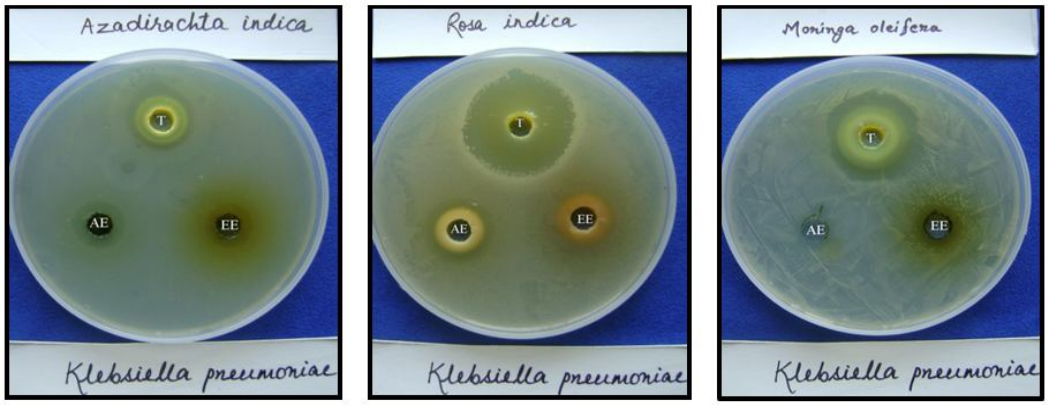

Figure 2: Antibiogram analysis of various plant extracts against *K.pneumonia*

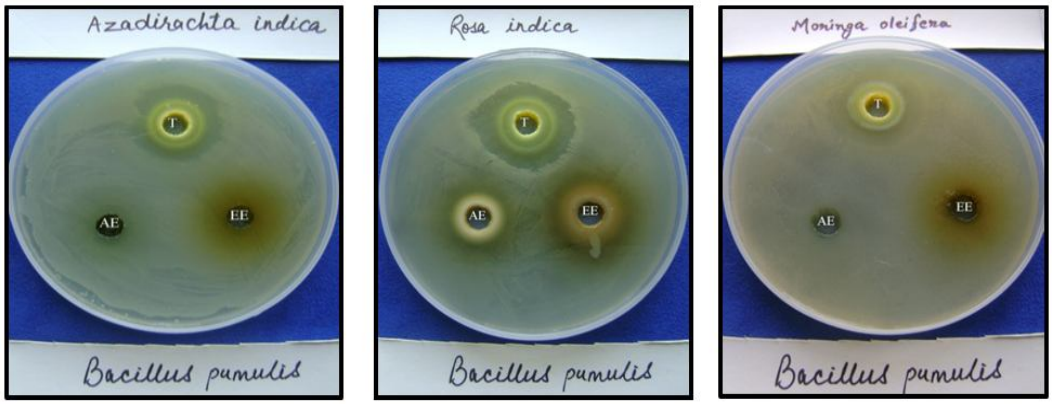

Figure 3: Antibiogram analysis of various plant extracts against *B.pumilis*

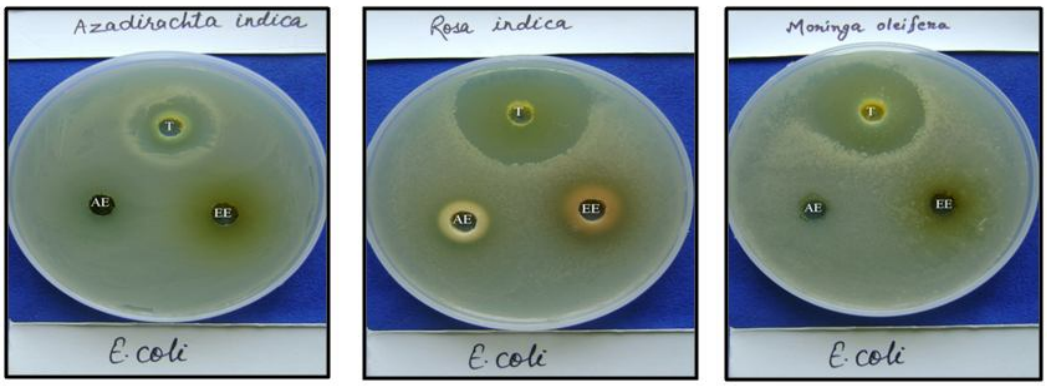

Figure 4. Antibiogram analysis of various plant extracts against *E.coli*

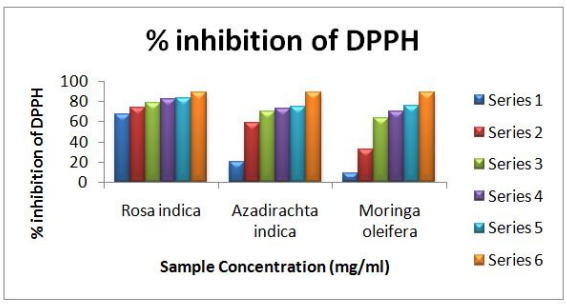

Figure 5. Comparative analysis of Free Radical Scavenging Activity of different plant extracts at different concentrations

Note: Series 1-5 = Plant Sample, Series 6 = Standard (L-Ascorbic acid), Series 1 = 0.2mg/ml, Series 2 = 0.4 mg/ml, Series 3 = 0.6 mg/ml, Series 4 = 0.8 mg/ml, Series 5 = 1.0 mg/ml, Series 6 = 1.0 mg/ml. Figure 5 and Table 5 showing that the DPPH scavenging activity was found maximum at 1.0 mg/ml concentration of ethanolic extract of Red Rose Petals i.e. 83.40%, which was quiet nearer to the standard we used i.e. L-Ascorbic acid while at the same concentration it was found to be 74.72% and 75.85% for Neem leaves and *Moringa* leaves respectively.

Total Phenolic Content (TPC) analysis

The contents of total phenolic compounds in crude ethanolic extracts obtained from Red rose petals, Neem leaves and Moringa leaves are presented in Table 7 while Table 6 and Figure 6 are showing the standard values of catechol. The results were reported as Catechol Equivalents (CE) mg/g extract.

Table 5. Comparative analysis of Free Radical Scavenging Activity of different plant materials at different concentrations

Plant Material	Material used	Concentration(mg/ml)	O.D. at 517nm	% inhibition of DPPH
L-Ascorbic acid	-	1.0	0.221	88.98
Rosa indica	Petals	0.2	0.658	67.19
		0.4	0.514	74.37
		0.6	0.432	78.46
		0.8	0.362	81.95
		1.0	0.333	83.40
Azadirachta indica	Leaves	0.2	1.591	20.68
		0.4	0.822	59.02
		0.6	0.599	70.14
		0.8	0.545	72.83
		1.0	0.507	74.72
Moringa oleifera	Leaves	0.2	1.819	09.32
		0.4	1.357	32.35
		0.6	0.723	63.95
		0.8	0.600	70.09
		1.0	0.485	75.82

Note-O.D. of control (DPPH in methanol) at 517 nm=2.006

Table 6. Standard values of Catechol at different concentrations

Standard	Concentration(mg/ml)	O.D. at 650 nm
Catechol	0.02	0.142
	0.04	0.290
	0.06	0.428
	0.08	0.616
	0.10	0.739

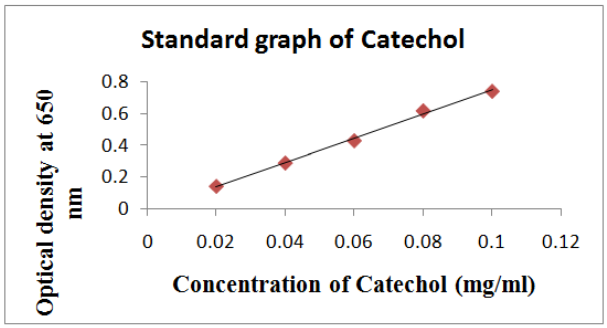

Figure 6. Standard graph of catechol

Table 7. Total phenolic content of ethanolic extracts of various plant samples under study

Plant material	Plant part used	Concentration (mg/ml) of plant extract	O.D. at 650 nm	Total Phenol (mg CE/g extract)
Rosa indica	Petals	1.0	0.687	96.00
A. indica	Leaves	1.0	0.534	74.00
M. oleifera	Leaves	1.0	0.622	83.00

Table 7 showing that the highest concentration of total phenol was 96 mg CE/g in the ethanolic extract of Red Rose petals at 1.0 mg/ml concentration whereas lowest i.e. 74 mg CE/g in ethanolic extract of Neem leaves at the same concentration.

Total Flavonoid Content (TFC)

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. These are well-known antioxidant constituents of plants and possess radical scavenging properties. (Miliauskas *et al.*, 2004). Total flavonoid compounds in crude ethanolic extracts obtained from Red rose petals, Neem leaves and Moringa leaves are presented in Table 9 while Table 8 and Figure 7 are showinh the standard values of quercetin. The results were reported as Quercetin Equivalents (QE) mg/g extract.

Table 8. Standard values of Quercetin at different concentrations

Standard	Concentration(mg/ml)	O.D. at 415 nm
Quercetin	0.02	0.224
	0.04	0.450
	0.06	0.666
	0.08	0.854
	0.10	1.054

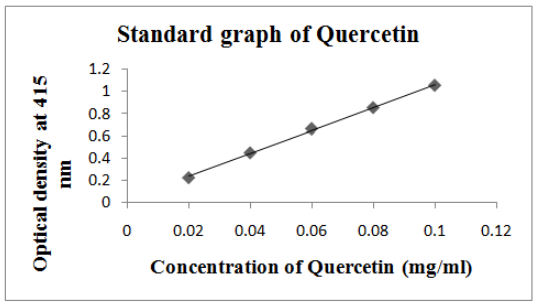

Figure 7. Standard graph of Quercetin

Table 9. Total flavonoid content of ethanolic extracts of various plant samples under study

Plant material	Plant part used	Concentration of plant extract (mg/ml)	O.D. at 415 nm	Total Flavonoids (mg QE/g extract)
Rosa indica	Petals	1.0	0.443	39
A. indica	Leaves	1.0	0.468	42
M. oleifera	Leaves	1.0	0.586	52

Table 9 is showing that the highest concentration of total flavonoids was 52 mg QE/g present in the ethanolic extract of moringa leaves at 1.0 mg/ml concentration, where as lowest i.e. 39 mg E/g in ethanolic extract of Red Rose petals at the same concentration. While in case of ethanolic extract of Neem leaves, the total flavonoids content was found to be 42 mg CE/g.

Photosynthetic Pigments (Chlorophyll A, B and Carotene)

Chlorophyll content

Chlorophyll is vital for photosynthesis, which allows plants to absorb energy from light. In this study, acetone extracts of Red rose petals, Neem leaves and *Moringa* leaves were analyzed to determine chlorophyll A and B content. The results observed are tabulated in Table 10.

Table 10. Comparative analysis of chlorophyll A and B content in the acetone extracts of various plants under study

Plant Material	Plant part used	O.D. at 662 nm	O.D. at 644 nm	Value of X		Chlorophyll content (mg/g dry weight)	
				Chl A (X_1)	Chl B (X_2)	Chl A	Chl B
A. indica	Leaves	1.046	0.430	9.808	4.349	0.490	0.217
M. oleifera	Leaves	1.732	1.072	15.884	14.743	0.794	0.737
Rosa indica	Petals	0.128	0.175	1.079	3.154	0.054	0.157

Chl A=Chlorophyll A, Chl B=Chlorophyll B

Table 11. Comparative analysis of carotene content in the acetone extracts of various plants under study

Plant Material	Plant part used	O.D. at 440 nm	Value of X	Carotene content (mg/g dry weight)
A. indica	Leaves	1.333	2.464	0.123
M. oleifera	Leaves	2.686	4.402	0.220
Rosa indica	Petals	0.392	0.706	0.035

Volume of acetone=10 ml, Weight of sample=200 mg

Table 10 showing the variable ranges of chlorophyll content found in various plant materials under study. On comparing the three plant materials, the chlorophyll content (both Chl A and Chl B) was found to be maximum in *Moringa* leaves i.e. Chl A=0.794 mg/g dry weight and Chl B=0.737 mg/g dry weight, and minimum in red rose petals i.e. Chl A=0.054 mg/g dry weight and Chl B=0.157 mg/g dry weight. Significant amount of chlorophyll was also found in Neem leaves.

Carotene content

Carotenes contribute to photosynthesis by transmitting the light energy they absorb from chlorophyll. They also protect plant tissues by helping to absorb the energy from singlet oxygen, an excited form of the oxygen molecule O_2 which is formed during photosynthesis. In this study, acetone extracts of Red rose petals, Neem leaves and *Moringa* leaves were analyzed to determine carotene content. The results observed are tabulated in Table 11.

Table 11 showing the variable ranges of carotene content found in various plant materials under study. The carotene content was found to be maximum in acetone extract of Moringa leaves i.e. 0.220 mg/g dry weight, while minimum in Red rose petals i.e. 0.035 mg/g dry weight. Neem leaves also showed significant amount of carotene content i.e. 0.123 mg/g dry weight.

Conclusion

Based on our results, it can be concluded that extracts of several plants and their different parts contain great amount of such compounds that have antibacterial and antioxidant activity. So that they can be used for the treatment of several infectious diseases caused by pathogenic microorganisms which are resistant against several available drugs. They also contain a remarkable amount of antioxidant agents which can prevent us from the highly damaging free radicles or reactive oxygen species formed through various metabolic pathways running in our body. Since the results are from one time study, this experiment may be repeated in order to confirm the findings. Further studies are required to understand the mechanism and the actual efficacy of these plant extracts.

REFERENCES

Akhmadieva AK, Zaichkina SI, Ruzieva R K, Ganassi EE. 1993. [The protective action of a natural preparation of anthocyanin (pelargonidin-3,5-diglucoside)]. Radio biologiia. 1993 May-Jun; 33(3): 433-5.

Bray, H.G., and Thorpe, W.V. (1954). Meth. Biochem. Anal. *J. Antimicrob. Chemother*. Vol. 1: 27-52.

Cai, Y., Luo, Q., Sun, M., and Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life science.* Vol. 74: 2157-2184.

Chan, E.W.C., Lim, Y.Y., and Chew, Y.L. (2007). Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia. *Food Chem.* Vol. 102, (4): 1214-1222.

Davis J (2009) Inactivation of the antibiotics and the dissemination of resistance genes. Science, 264: 375-82.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem* 2006, 97; 654–660.

Johnson C: Clinical Perspectives on the Health Effects of *Moringa oleifera*: A Promising Adjunct for Balance Nutrition and Better Health. La Canada, CA, KOS Health Publications, 2005.

Miliauskas, G., Venskutonis, P.R., Van Beek, T.A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.* Vol. 85: 231-237.

Mitra, C. R., *Neem*, Dr M. S. Patel, Indian Central Oilseeds Committee, Hyderabad, 1963, pp. 69–94.

Morena M, Cristol J, Canaud B. 2000. Why hemodialysis patients are in a prooxidant state? What could be done to correct the pro/antioxidant imbalance. Blood Purif 18:191–199

Nickon, F., Saud, Z.A., Rehman, M.H. and Haque, M.E. (2003): In vitro antimicrobial activity of the compound isolated from chloroform extract of M. oleifera Lam. Pak. J. Biol. Sci. 22:1888 – 1890.

Pitchaon, M., Suttajit, M., and Pongsawatmani, R. (2007). Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. *Food Chem.* Vol. 100: 1409-18.

Pokorny, J., Yanishlieva, N., and Gordon, M. (2001). Antioxidants in food. *Practical Applications,Cambridge Woodhead publishing limited*. Vol. 72, (5): 145-71.

Ruckmani, K., Kavimani, S., Anandan, R. and Jaykar, B. (1998): Effect of Moringa oleifera Lam on paracetamol – induced hepatoxicity. Indian J. Pharm. Sci. 60:33 – 35.

Saric, M., Kastrori, R., Curie, R., Cupina, T., and Gerie, I. (1976). Chlorophyll determination. Univ. Unoven Sadu Parktikum is fiziologize Bibjoke, Beagard, Hauncna, Anjig. 215. *Current Science*. Vol. 5: 23-25.

Siddiqui, S., *Curr. Sci*., 1942, 11, 278–279.

Sreelatha S, Padma PR: Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods Hum Nutr 64:2009; 303–311

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