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Chemical Composition and Biological Activities Guided Assessment of Essential Oils of Two Rutacea Species

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The volatile oil obtained by hydro-distillation of matured peels of *Citrus reticulata* and leaves of *Murraya koenigii,* belonging to the family Rutacea were subjected to GC-MS analysis for their chemical characterization. Major components were found to be Limonene (92.83%) in *C. reticulata* and Eugenol (81.61%) in *M. koenigii.* Both oils showed good antioxidant activity by scavenging DPPH radical (IC_{50} = 24.77 and 26.68 μg/ml) and inhibiting linoleic acid (70.33 and 82.78 %) for both *C. reticulata* and *M. koenigii,* respectively. Antimicrobial activity of oils was accomplished using disc diffusion method followed by minimum inhibitory concentration (MIC) against various pathogenic bacteria and fungi. Fungal strains were found to be more sensitive against both of the oils. Minimum hemolysis (0.08 & 0.34%) was observed at 0.5 mg/mlin case of *M. koenigii* against both human and bovine erythrocytes, respectively.

Key words: *Citrus reticulata*, *Murraya koenigii*,GC-MS, Antioxidant activity, antimicrobial activity, Hemolytic Assay

Cellular metabolism results in generation of toxic byproducts including free radicals of oxygen and nitrogen which are much reactive species. These free radicals show adverse effect on human biological system in the situation known as oxidative and nitrosative stress as the production of ROS/RNS occurs beyond the normal limit[1]. The biological units like proteins, lipids, DNA etc. are damaged and deteriorated by overproduction of ROS which lead to a large number of diseases in human beings. The primary ROS produced in biological system is superoxide radical ($O_2^{\bullet-}$) which is highly reactive due to presence of unpaired electron and starts reacting with biological molecules to produce secondary radicals likeHydroxyl radical ($OH^{\bullet}$), peroxynitrate ($ONOO^{\bullet}$), peroxyl radical ($LOO^{\bullet}$) and also the singlet oxygen ($O^{\bullet}$).

Several factors are responsible for the decay of food products like peroxidation of fatty food together with contamination by various pathogens which not only cause poisoning of food but also responsible for the various diseases and economic losses as well. Therefore it becomes imperative to safeguard the food stuff by avoiding their spoilage and to maintain their shelf life at different stages of storage and distribution. Extended shelf life is desired in the food industries

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as they require extended time for transportation and distribution of food products to far off areas. A number of steps have been taken in the traditional cold distribution of such delicate food products all over the world but this does not assure 100% protection of such foods. Alternatives to this include pulsed light,antimicrobial agents,inert gases and different radiations and have become common preservative techniques these days in food industry.[2,3] Variety of synthetic preservatives are also available in the market which are effective but their toxicity towards human health has always been a great concern.[4] These include bulylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG) which have been used since decades[5] But their use has now been limited due to their carcinogenic nature.

Therefore it has become dire need of time to explore the natural resources like extracts and essential oils of herbs and spices as potential food preservatives[6] Value addition of food stuff by introducing antioxidants is an efficient alternative for retarding oxidation process[7] The natural and non-carcinogenic antioxidants are more effective, safer and reliable towards human health and have attracted interest of food scientists. Various compounds extracted from medicinal and aromatic plants have exhibited strong antioxidant potential[8,9] Essential oils extracted from a variety of herbs and spices have shown strong potency as food preservative, natural therapies and alternative medicines together with their pharmacological uses in various liver, stomach and cancer related problems.[10]

Rutacea, also known as citrus family, is the family of flowering plants consisting of about 170 genera and 15000 species[11] found extensively in various mild and humidpartsof Australia and Southern Africa. There are some 11 genera and 27 species located in Pakistan, which are strongly fragrant due to presence of essential oil[12] The principle genera of Rutaceae include Citrus, Zanthoxylum, Ruta, Ptelea, Murraya and Fortunella.Citrus fruits, bitters, curry leaves and various decorative plants belong to the same family.

The genus Citrus includes about 17 species, used for making desserts and has significant economical value due to presence of a variety of aromatic compounds in essential oil.[13,14] The tree of *Citrus reticulata* is thorny, small in size and top is denser having slim branches. Liquors are being flavored using the peel of *C. reticulata*. Various studies have reported the chemical characterization and anti cancer activity of *C. reticulata* essential oil.[15,16,17]*Murraya koenigii*, widely distributed in Asian countries, is a round 6m high shrub having short stalk, brownish grey bark with a thick shadowy top.[18] Essential oil from leaves are used for flavoring purposes in a variety of dishes including curries, fish, meat and egg along side different soaps and cosmetic aromatherapy[19] Traditionally it has been used in treatment of diarrhea, purification of blood, body and kidney pains.[20-22]

The urgebehind present work was to assess the essential oil potential of two Rutacea species i.e. *C. reticulata* and *M. koenigii* asantioxidant and antimicrobial agents by hydro distillation from peels and leaves respectively.

MATERIALS AND METHODS

Plant material

The sampling of *C. reticulata* was done in district Chakwal and peels were separated out where as *M. koenigii* was cultivated in the experimental farm of Kamal laboratories, Sukho, Rawalpindi (Pothohar region).

Essential Oil Distillation

Extraction of essential oil was done by taking the weighed amount of sample in a Clevenger type apparatus and the process of hydro distillation was run for about 3 hrs. The separation of oil and water mixture was followed by the drying process of oil over anhydrous sodium sulphate. Oil was filtered to separate the salt and stored in colored sealed glass bottles at 4^{o}C.[23]

Physical analysis

The various physical parameters like color, refractive index and specific gravity of essential oils were determined using methods given by Guenther[24] Digital refractometer (ATAGO Digital refractometer, R 3261) was used to measure the refractive index of oil samples.

Analysis by Gas chromatography–mass spectrometry

The volatile components of essential oils were analyzed using gas chromatograph, HP 5890-series II equipped with mass spectrophotometer,

MSD 5972 system. Column was Phenomenex ZB-5MS (30m length x 0.25mm ID x 0.25µm film thickness), with carrier gas, Helium, at the flow rate of 0.7ml/min. The oven temperature programming was done as follows; 40°C for 1 min, 40–240°C @ 8°C/min and held isothermal for 2 min, then 240-300°C @ 10°C/min. The injection port was maintained at 250°C whereas detector at 275°C. 1µL of 1% solution in hexane was injected; HP 5972 recording at 70eV with mass range 50–550amu. Chem Station was used as software to handle mass spectra. NIST 98 NIST/EPA/NIH mass spectral library was used for the identification of oil components as well as by comparison of their retention indices with literature data.[25,26] Retention indices were determined relative to the retention times of a series of n-alkanes (relative to $C_9 - C_{28}$ on the same column).

Antioxidant Activity

The ability of essential oils to scavenge DPPH stable radical was used to assess the antioxidant potential with slight modifications[27] and the antioxidant activity was expressed as IC_{50} (µg/ml); the amount which was responsible for 50% inhibition of free radical. Lesser value of IC_{50}attributed the higher potential of essential oil towards antioxidant activity.[28]Antioxidant activity of *C. reticulata* and *M. koenigii* essential oils was also evaluated using inhibition of linoleic oxidation system with modifications.[29,30]Similarly another assay used to test the antioxidant activity was bleaching of β-carotene/ linoleic acid emulsion system which was used with slight modiûcations.[31]Positive control used was Butylated hydroxytoluene (BHT) in all the above mentioned assays.

Antimicrobial Activity

The antimicrobial activity of oils was tested against variety of microorganisms including four Gram-positive bacteria;*Bacillus subtilis* (*B. subtilis*), *Lactobacillus rhamnosus (L. rhamnosus)*, *Staphylococcus aureus* (*S. aureus*), *Streptococcus mutans (S. mutans)*, two Gram-negative bacteria: *Escherichia coli* (*E. coli*) and *Pasteurella multocida* (*P. multocida*), and four pathogenic fungi: *Alternaria alternata* (*A. alternata*), *Aspergillus flavus* (*A. flavus*), *Aspergillus niger* (*A. niger*), and *Ganoderma lucidum* (*G. lucidum*). The pure microbial strains were obtained from Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan and Medicinal & Aromatic Plant Laboratory, Stockbridge School of Agriculture, University of Massachusetts, MA, USA. Antimicrobial activities of oils were tested against selected microbial strains using disc diffusion assay by following the methodology of National Committee for Clinical Laboratory Standards[32] Disc without sample was used as negative control while Rifampicin (30µg/disc) and Terbinafine (30 µg/disc) applied discs were used as positive control for bacteria and fungi, respectively. Diameter of growth inhibition zones (mm) was the indicator for antimicrobial activity.Clinical Laboratory Standard Institute[33] methodology was used to calculate the minimum inhibitory concentration (MIC) in 96-well plates. The plates were read using an automatic ELISA micro plate reader (Bio-Tek-USA) adjusted to 620 nm from where information about an increase or decrease in microbial growth was obtained. The 50% inhibition in microbial growth, recorded as MIC was obtained by plotting control against lowest concentration of sample.

***In vitro* Hemolytic assay for measurement of Cytotoxicity**

The hemolytic potential of essential oils was investigated using the method given by Sharma and Sharma[34] in which (B^-) RBC suspension was mixed with essential oil samples at three different concentrations 0.5 mg/ml, 5 mg/ml and 10 mg/ml in DMSO.[35] 100% hemolysis (positive control), was obtained by mixing Triton X-100 (in PBS) with RBC suspension.

RESULTS AND DISCUSSION

The yield (g/100g of dry plant materials) of essential oils of *C. reticulata* and *M. koenigii* was found to be 0.91 and 1.12%, respectively as listed in Table 1. The essential oil obtained from *C. reticulata* was colorless with strong aromatic smell and refractive index 1.355 whereas in case of *M. koenigii* it was found to be greenish yellow in color with refractive index 1.357. Due to low density both essential oils were collected on the surface of water during hydro distillation process with specific gravity 0.81 and 0.95 for both *C. reticulata* and *M. koenigii*, respectively. In another study yield of essential oil from *C. reticulata* was recorded as 0.6 ml/100g peel.[36]*Citrus limettioides* essential oil was

tested for its various physical parameters including color,odor, specific gravity, refractive index and optical rotation.[37]Similarly, 0.12 % yield of colorless essential oil was obtained from dry leaves of *M. koenigii* with typical odor and taste.[21]

The complete profile of chemical composition of *C. reticulata* essential oil obtained by GC-MS is listed in Table 2. Total 27 compounds were identified making 98.57 % of the total oil composition. Limonene (92.83 %) was found to be

Table 1. Physical properties of *Rutacea* essential oils

Parameter	*Citrus reticulata*	*Murraya koenigii*	BHT
Colour	Colourless	Greenish yellow	-
Yield (%)	0.91±0.03	1.12±0.1	-
Refractive Index (30 °C)	1.355±0.003	1.357±0.002	-
Specific Gravity	0.81±0.04	0.95±0.01	-
Antioxidant activity DPPH, IC_{50} (µg/mL)	24.77±0.78	26.68±2.72	3.46±0.3

Values are mean ± standard deviation of three samples of each *Citrus reticulata* and *Murraya koenigii* analyzed individually in triplicate.

Table 2. Chemical composition of *Citrus reticulata* essential oil

Components[1]	RI[2]	% age	Mode of Identification[3]
alpha.-Pinene	934	0.19	RT, RI, MS
Sabinene	972	0.13	RT, RI, MS
β-Pinene	978	0.15	RT, RI, MS
beta.-Myrcene	994	0.69	RT, RI, MS
3-Carene	1011	0.24	RT, RI, MS
Limonene	1033	92.83	RT, RI, MS
β-Ocimene	1044	0.22	RI, MS
γ-Terpinen	1072	0.21	RT, RI, MS
Linalool	1096	0.31	RT, RI, MS
α- Terpinolen	1187	0.14	RT, RI, MS
Citronellol	1228	0.18	RT, RI, MS
3-p-Menthene	1234	0.20	RI, MS
Linalyl alcohol	-	0.15	RT, MS
Neryl acetate	1344	0.19	RT, RI, MS
Eugenol	1356	0.21	RT, RI, MS
Copaene	1366	0.19	RT, RI, MS
Patchoulane	1378	0.24	RI, MS
β-Terpineol acetate	-	0.17	RT, MS
Aromadendrene	1440	0.25	RI, MS
Germacrene D	1451	0.22	RT, RI, MS
Isocaryophyllene	-	0.17	RT, MS
alpha.-caryophyllene	1454	0.19	RT, RI, MS
trans-Linalolxide	-	0.26	RT, MS
alpha.-Farnesene	1509	0.18	RT, RI, MS
β-Gurjurene	-	0.20	RT, MS
δ-Cadinene	-	0.21	RT, MS
γ-Eudesmol	1623	0.24	RT, RI, MS
Total (27)		98.57	

[1]Compounds are listed in order of elution from a ZB-5MS column.
[2]Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.
[3]RT = identification based on retention time, RI = Identification based on retention index,
MS= identification based on comparison of mass spectra

the major component of total oil composition. Our results were in good agreement with those reported by Chutia *et al.*,where fresh and matured fruit of *C. reticulata* Blanco was collected from Jorhat, Assam (India).[36] The freshly collected peels were subjected to essential oil extraction and key chemical compounds were found to be limonene (46.7 %)and geranial (19.0 %). In another study[38]citrus peels of some 13 species were collected from Jeju island (Korea) and essential oils extracted was subjected towards GC-MS analysis. Limonene (82.43 %) and α-terpinene (6.83 %) were the major components among others. Hamdan *et al.* tested two citrus species;*C. jambhiri and C. pyriformis* collected from Egypt.[39] The chemical characterization of their essential oil also evidenced limonene to be the most abundant compound 92.48 and 75.56 % for *C. jambhiri and C. pyriformis*,respectively.

The data for chemical composition of the essential oil from *M. koenigii* is listed in Table 3, where 25 compounds were identified making 98.43 % of the total oil. The prominent compounds were eugenol (81.61 %), benzyl benzoate (7.13 %) and myristicin (1.0 %). Ningappaa *et al.* collected *M. koenigii* leaves from Kota Belud, Sabah (Malaysia) and subjected its essential oil for chemical characterization by GC-MS and found β-caryophyllene (19.50 %), ±-humulene (15.24 %), *p*-cymen-8-ol (10.31 %) as major components.[21]

The essential oils of *C. reticulata* and *M. koenigii* were assessed for their antioxidant potential using DPPH assay. DPPH• (Purple radical) is reduced to DPPH-H (yellow) during the process by taking electron or hydrogen atom from the tested essential oil. The results of radical scavenging by

Table 3. Chemical composition of *Murraya koenigii* essential oil

Components[1]	RI[2]	% age	Mode of Identification[3]
Cyclofenchene	896	0.41	RI, MS
Cumene	926	0.22	RT, RI, MS
Camphene	955	0.46	RT, RI, MS
β-Thujene	970	0.38	RT, RI, MS
Sabinene	972	0.34	RI, MS
alpha.-Phallendrene	1005	0.28	RT, RI, MS
α-Terpinene	1020	0.29	RT, RI, MS
γ-Terpinen	1072	0.43	RT, RI, MS
1,5-cyclooctadiene, 1,5-dimethyl	-	0.11	RT, MS
Linalyl alcohol	-	0.65	RT, MS
1-Phenyl-1-methylbutane	-	0.26	RT, MS
3-p-Menthene	1234	0.3	RT, RI, MS
Linalyl butanoate	-	0.33	RT, MS
Isohomogenol	-	0.9	RT, MS
α-Terpinyl acetate	1350	0.44	RT, RI, MS
α-Cumyl hydroperoxide	-	0.19	RT, MS
Eugenol	1356	81.61	RT, RI, MS
Linalyl propanoate	-	0.37	RT, MS
Cinnamic acid	1387	0.52	RT, RI, MS
Vinyl Cyclohexene dioxide	-	0.24	RT, MS
alpha.-himachalene	1451	0.44	RI, MS
Myristicin	1521	1.00	RI, MS
Eugenyl acetate	1524	0.84	RT, RI, MS
trans-γ-Caryophyllene	-	0.18	RT, MS
Benzyl Benzoate	1764	7.13	RI, MS
Total (25)		98.43	

[1]Compounds are listed in order of elution from a ZB-5MS column.
[2]Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.
[3]RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

examined essential oils were presented in terms of 50% scavenging (IC_{50}) and found to be 24.77 and 26.68 µg/ml for both *C. reticulata* and *M. koenigii* oils, respectively as shown in Table 1.While in case of BHT, synthetic antioxidant, it was 3.46 µg/ml. Antioxidant capability of oils was also tested using linoleic acid, an unsaturated fatty acid having ability to form peroxides which could oxidize Fe^{+2} to Fe^{+3} leading it towards formation of complex with SCN^{-} present in reaction medium. The complex could be measured quantitatively using spectrophotometer at 500 nm. Percent inhibition in linoleic acid system by both oils at three different concentrations is shown in the Figure 1 which revealed maximum inhibition 82.78 and 70.03 % at concentration 50mg/ml for both *M. koenigii* and *C. reticulata* samples respectively which decreased with the decrease in concentration that could be due to decrease in bioactive compound concentration. The inhibition shown by BHT was

Table 4. Antimicrobial activity of *Rutacea* essential oils

Tested organism	Essential oils		Rifampicin	Terbinafine
	Citrus reticulata	*Murraya koenigii*		
Inhibition zone (mm)				
B. subtilis	19.0±1.6	5.2±0.6	10.8±2.1	-
L. rhamnosus	14.3±0.1	12.4±0.6	15.5±0.8	-
S. aureus	6.1±1.0	14.3±0.7	15.1±1.0	-
S. mutans	6.8±0.4	13.4±0.2	13.4±0.7	-
E. coli	5.3±0.4	9.3±0.5	9.6±1.3	-
P. multocida	7.4±0.1	10.4±0.5	11.6±1.5	-
A. alternata	5.5±0.3	18.3±1.2	-	14.2±0.8
A. flavus	20.1±1.4	11.6±0.5	-	11.4±1.4
A. niger	19.6±2.1	11.7±1.2	-	21.7±1.2
G. lucidum	3.6±0.3	10.7±0.3	-	9.1±0.4
Minimum inhibitory concentration (MIC) mg/mL				
B. subtilis	1.41±0.1	4.06±0.3	1.72±0.2	-
L. rhamnosus	2.03±0.1	2.34±0.1	2.81±0.3	-
S. aureus	3.75±0.3	2.03±0.1	2.19±0.2	-
S. mutans	4.06±0.3	2.34±0.2	2.03±0.1	-
E. coli	4.48±0.2	2.81±0.2	2.34±0.2	-
P. multocida	5.62±0.3	2.5±0.1	2.03±0.1	-
A. alternata	5.0±0.3	1.56±0.1	-	2.34±0.2
A. flavus	1.17±0.1	2.34±0.2	-	2.81±0.2
A. niger	1.25±0.1	2.34±0.1	-	0.94±0.1
G. lucidum	5.62±0.4	2.5±0.2	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Citrus reticulata*, analyzed individually in triplicate.

Table 5. Cytotoxicity (% hemolysis) of *Rutacea* essential oils

Essential oil	Concentration	Human erythrocytes (%)	Bovine erythrocytes (%)
Citrus reticulata	0.5mg/mL	3.12	2.33
	5mg/mL	5.90	5.22
	10mg/mL	9.96	8.84
Murraya koenigii	0.5mg/mL	0.08	0.34
	5mg/mL	1.80	3.14
	10mg/mL	8.26	9.53
	PBS	0.00	0.00
	Triton X-100	100	100

85.10 % which indicated that oils showed comparable activity with synthetic antioxidant. The inhibition in reduction of β-carotene by peroxyl radical (LOO•) formed by oxidation of linoleic acid due to presence of antioxidant was also examined by depletion of color of solution using spectrophotometer at 470 nm. Antioxidant activity of *C. reticulata* and *M. koenigii* essential oil in terms of β-carotene bleaching is shown in Figure 2. The maximum color depletion in case of control proved its least activity,whereas oils having antioxidant potential, exhibited less color depletion. Three different concentrations of oils were used where minimum depletion and maximum activity was observed at 6 mg/ml. The color was least faded in case of BHT showing its maximum activity. Hamdan *et al* used DPPH assay to evaluate activity of two citrus species[39] IC50 values were calculated for *C. pyriformis and C. jambhiri* essential oils as 28.91 and 37.69 mg/ml, respectively. Baik[38]evaluated 14 citrus species collected from various regeions of Korea for their antioxidant potential. Similarly the antioxidant activity of *M. Koenigii* leaf extracts prepared in different solvents were evaluated[40] Rao *et al.* extracted two alkaloids, from the *M. koenigii* leaves, Mahanimbine and koenigine, which exhibited a high degree of radical scavenging ability.[19]

Table 4 shows the antimicrobial activity of *C. reticulata* and *M. koenigii* essential oils against various bacterial and fungal strains. In case of some fungi and bacteria, results were even quite better as compared with antibiotics.*C. reticulata* showed larger inhibition zones in disc diffusion method (5.3-19.0 and 5.5-20.1 mm) with smaller MIC values (1.41-5.62 and 1.17-5.62 mg/ml) against a variety of bacteria and fungi, respectively. Among bacterial strains best activity was against *B. subtilis* (Gram positive) with maximum inhibition zone of 19.0 mm followed by minimum MIC value of 1.41 mg/ml. The results were compared and found quite better than that of antibiotic, Rifampicin, which exhibited the inhibition zone 10.8 mm and MIC 1.72 mg/ml. *A. flavus*,among all the tested fungi,proved to be most sensitive with largest inhibition zone 20.1 mm and minimum MIC value 1.17 mg/ml which was found much improved in comparison with antibiotic, Terbinafine, with inhibition zone 11.4 mm and MIC 2.81 mg/ml. Vasudeva and Sharma tested essential oil of *Citrus limettioides* against various microbes and maximum activity in case of bacteria was found against *Propionibacterium acnes* with MIC value 3.12 µL/ml, while in case of fungal strains against *Aspergillus niger* with MIC 6.25 µL/ml.[24]*M. koenigii* showed larger zones (5.2-14.3 and 10.7-18.3 mm) with lesser MIC values (2.03-4.06 and 1.56-2.5 mg/ml) against bacterial and fungal strains, respectively. *S. aureus* (Gram positive) showed maximum activity amongst all the tested bacteria with largest zone of inhibition; 14.3 mm and minimum MIC value 2.03 mg/ml. These results were much closer to that of antibiotic, Rifampicin, whose zone of inhibition was 15.1 mm and MIC 2.03 mg/ml. Among fungi, *A. alternata* wasthe most sensitive with largest zone 18.3 mm and MIC value 1.56 mg/mlthat was quite better than that of antibiotic, Terbinafine, with zone 14.2 mm and MIC 2.34 mg/ml. In another study essential oil of *M. koenigii* was tested against different bacterial strains and

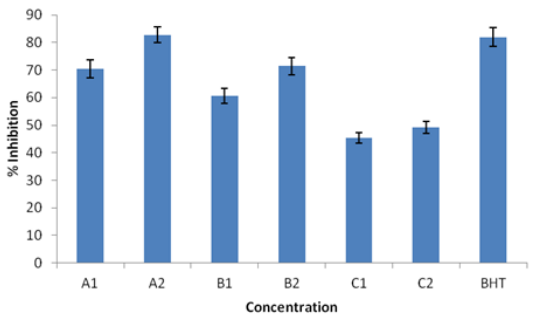

Fig. 1. Antioxidant activity of essential oils: A, 50mg/mL; B, 30mg/mL; C, 10mg/mL; 1, *Citrus reticulata*; 2, *Murraya koenigii*; BHT, Butylated hydroxytoluene 200ppm

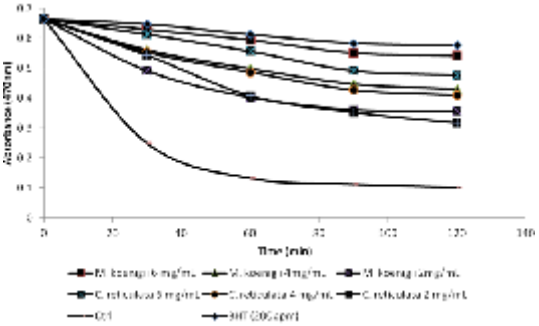

Fig. 2. Antioxidant activity of *Citrus reticulata* and *Murraya koenigii* essential oils measured by bleaching of β-carotene-linoleic acid emulsion

zone of inhibition was ranged between 10.00 mm to 18.50 mm, with greater activity for*Streptococcus pneumonia*.[21] The minimum activity was against *Psedomonas aeruginosa* and *Klebsiella pneumoniae* with MIC value; 25.00 µg/ml while 200.00 µg/ml was the MBC against *S. pneumoniae*.

Cytotoxicity of *C. reticulata* and *M. koenigii* essential oils was investigated on human and bovine erythrocytes. Essential oils in three different concentrations were used and % hemolysis was calculated and presented in table 5. Results indicated that both the erythrocytes showed increased sensitivity with the increase in essential oilconcentration. *C. reticulate,*for bovine erythrocytes, exhibited hemolysis i.e. 8.84 % at 10 mg/ml concentration and 2.33 % at 0.5 mg/ml concentration, while in case of human erythrocytes it was 9.96 % at 10 mg/ml concentration and 3.12 % at 0.5 mg/ml. Maximum hemolysis against bovine erythrocytes shown by*M. koenigii* was 9.53% at 10 mg/ml concentration and the minimum was 0.34 % at 0.5 mg/ml, while with human erythrocytes it was 8.26 % at 10 mg/ml concentration and 0.08 % at 0.5 mg/ml. In another study carbazole alkaloid extracted from *M. koenigii* showed considerable cytotoxic potential.[41]

CONCLUSION

The essential oil of *Citrus reticulata* and *Murraya koenigii*, belonging to the family of Rutacea, were chemically characterized by GC-MS indicated that Limonene (92.83%) and Eugenol (81.61%) were the major components, respectively. Good antioxidant activity was found by scavenging DPPH radical (IC_{50} = 24.77 and 26.68 µg/ml) and inhibiting linoleic acid (70.33 and 82.78 %) for both *C. reticulata* and *M. koenigii*, respectively. Antimicrobial activity showed that fungal strains were more sensitive against the both oils. The results were found to be promising as compared to standards used.

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