

COMPARATIVE STUDY ON PHARMACOLOGICAL ACTIVITIES OF ESSENTIAL OILS OF SELECTED AROMA PLANTS

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ABSTRACT

Essential oils have high Industrial, economic and therapeutic values. Keeping the abundant availability of aroma and medicinal plants still to be explored for their importance, the present study has been undertaken to carry out phytochemical and pharmacological examination of selected plants with a focus on essential oils and the solvent extracts of plants. The chemical constituents of the isolated oil by hydrodistillation, concretes and absolutes were determined by GC-MS analysis and the details are presented comparatively. The pharmacological examination carried out for the oil, anti-microbial, anti-inflammatory and anti-oxidant activities by in vitro methods.

KEYWORD: Hydrodistillation, anti-microbial, anti-inflammatory, anti-oxidant activity, in vitro method, GC-MS analysis, essential oil.

Aim of the present study

Survey of literature revealed a few studies on the chemical composition and pharmacological aspects of aroma plants and almost all of them deal with the composition of the essential oil. Considering this, the present work has been designed with the following objectives:

- ❖ To isolate the essential oil by hydrodistillation of aroma plants.
- ❖ To carry out pharmacological examination of the oils for their anti-oxidant, anti-inflammatory and anti-microbial activity by in vitro methods.
- ❖ To study the CNS activity of polianthes tuberosa.

INTRODUCTION

Essential oils usually have the odorous or flavour of the plant material from which they are obtained and are used to make perfumes and flavourings which consist of a complex mixture of terpenes, sesquiterpenes and oxygenated sesquiterpenes", (Ullmann's Encyclopedia of Industrial Chemistry, Weinheim^[1]). Essential oils can also contain diterpenes and some specific compounds which can not be classified as belonging to any of the above mentioned compound families. Other compounds extracted from vegetable matter can be found in concretes, absolutes and oleoresins. Oleoresins are fatty acids and fatty acid methyl esters (FAMES), colouring matters (β -carotene). coumarins, psoralens, sterols and flavones.^[2] Essential oils are natural aromatic compounds found in seeds, bark, stem, roots, flowers, and other parts of the plants. Against predators, diseases and play a role in plant pollination.^[3]

Essential oils are non- water based phytochemicals made up of volatile aromatic compounds. Although they are fat soluble, they do not include fatty lipids or acids found in vegetable and animal oils.^[4] Essential oils are very clean, almost crisp, to the touch and are immediately absorbed by the skin. Pure, unadulterated essential oils are semi-transparent and ranges from crystal clear to deep blue colour. In addition to their benefits to plants and being beautifully fragrant to people, essential oils have been used throughout the history for their medicinal and therapeutic paybacks. Modern scientific study trends toward more holistic approaches to wellness are driving a revival and discovery of essential oil health applications. Commercially, essential oils are used in three primary ways as

- ❖ Flavours (bakery goods, candies, confectionaries, meat, pickles, and soft drinks).
- ❖ Pharmaceuticals (dental products and a wide but fading group of medicines).
- ❖ Odorants (cosmetics, perfumes, soaps, detergents, and miscellaneous industrial products ranging from animal feed to insecticides and paints).

Various sources of essential oils

Essential oils may be formed by the hydrolysis of certain glycosides or directly by protoplasm or by decomposition of the resinogenous layer of the cell wall. Inside the vegetable cells, the essential oils are contained in the "*vacuoles*", Cavities of roundish form bound by a single membrane, the *tonoplast* that contains an aqueous solution full of a juice, the "*vacuolar juice*". The vacuole is a cellular organelle, probably originating from the endoplasmic reticulum, into which are poured the "*secondary products*" or the products of refusal of the metabolism. Essential oils are generally derived from one or more plant parts,

such as flowers (e.g. Rose, Jasmine, Carnation, Clove, Mimosa, Rosemary, Lavander), leaves (e.g. Mint, Ocimum spp., Lemongrass, Jamrosa), leaves and stems (e.g. Geranium, Patchouli, Petitgrain, Verbena, Cinnamon), bark (e.g. Cinnamon, Cassia, Canella), wood (e.g. Cedar, Sandal, Pine)^[6], roots (e.g. Angelica, Sassafras, Vetiver) Saussurea, Valerian, seeds (e.g. Fennel, Coriander, Caraway, Dill, Nutmeg), Fruits (Bergamot, Orange, Lemon, Juniper), rhizomes (e.g. Ginger, Calamus, Curcuma, Orris)^[5] and gums or oleoresin exudations (e.g. Balsam of Peru, Styrax, Myrrh, Benzoin).

Biological functions of the essential oils

It is thought that in living plants, the essential oils develops a reserve function to feed and this can be proved by the fact that essence plants placed in the dark can be observed almost complete disappearance of fragrant principles that would be used to the placed of the reserve compounds. It is also thought that essential oils have a notable importance in pollination, in that they attract the insects and thus promoting pollination or that they have an important role in creating some barriers of protection against the predating animals.^[7]

Extraction of essential oils

Hydrodistillation

Majority of essential oils are produced by the process of steam distillation, which is the main method for flavour extraction. This is also the official standard method for extracting essential oils for quality control. In this method the distillation procedure is closely defined closable products, according to the reference method indicated in the standard is done with steam distillation, collection of the distilled products in xylene (solvent). During hydrodistillation the essential oil components form an azeotropic mixture with water. Most of the essential oils do not mix well with water in the liquid phase and hence after condensation, they are separated by decantation. The distillation period can take about 15 – 30 min or longer. The extraction period influences not only the yield but also the extract composition. Hydrodistillation can be achieved by Clevenger apparatus where the material to be extracted is immersed in water, which is then boiled or by steam distillation where the steam passes through a bed of the material to be extracted. In both methods, vapours of the volatile components are carried by the steam to a condenser. On condensation, oil-rich and water-rich layers are formed. These are separated by decantation. During both forms of hydrodistillation, the sample is exposed to temperatures close to 100° C, which can change the thermolabile components. Prolonged heating in contact with water can lead to hydrolysis

of esters, polymerization of aldehydes, or decomposition of other components. Hydrodistillation at elevated pressures is used for plants whose essential oils are difficult to extract. However, due to the high temperatures used, the danger of decomposition is even higher. Hydrodistillation at reduced pressure is better because lower temperatures are used in this case.

Pharmacological studies of essential oil

Antioxidants

Substances which hold back the oxidation processes by inactivating or scavenging free radicals are termed as antioxidants. Antioxidants are supposed to reduce the oxidative damage caused by reactive oxygen species, reactive nitrogen species and reactive chlorine species. Antioxidants should be non-toxic, relatively inexpensive, effective and should not alter the quality of end products.^[8]

Antimicrobials

The main features of all essential oils-beside the fragrance are their antimicrobial property. All essential oils have been proved to possess both antibacterial and antifungal activity. The effectiveness differs on the oil and is strictly linked with the oil chemical composition, and it is always dose dependent. Some essential oils exhibit strong antioxidant properties also. Both antioxidant and antimicrobial properties of essential oils are important for the therapeutic application of essential oil bearing plants that are used in medicine and essential oils especially used in aromatherapy.^[9,10,11]

Table 1: The comparative classification of aroma plants.

	VWRO	CRRO	PTC
Botanical name	Valeriana wallichii DC	Cyperus rotundus L.	Polianthes tuberosa L.
Family name	Valerianaceae	Cyperaceae	Asparagaceae
Kingdom	Plantae	Plantae	Plantae
Division	Magnoliophyta	Magnoliophyta	Magnoliophyta
Class	Magnoliopsida	Liliopsida	Monocotyledons
Order	Dipsacales	Poales	Asparagales
Family	Valerianaceae	Cyperaceae	Asparagaceae
Genus	Valeriana	Cyperus	Polianthes
Species	Valeriana wallichii	Cyperus rotundus	Polianthes tuberosa
Popular names	Valerian jatamansi, Taggar, great wild	Coco Grass, Purple Nut Sedge, Rednut Sedge, Mustaka.	Tuberose, Ivory flower
Vernacular name (Tamil)	Valerian Phu, Tagari	Accm, Avittam, Muthakasu, Korai kilangu.	Nila sampangi
Habitat	Temperate zone of North Western Himalayas	Weed found all over India	Friable, sandy soil

*Valeriana wallichii***Chemical examination of VWRO**

V. wallichii roots (1.0 kg) were extracted using hydrodistillation method and the essential oil (VWRO) was collected after 4 hours and stored at 4°C until further use (Yield: 1.80%).

Table 2: The details of the chemical constituents of VWRO.^[14]

Chemical constituent	VWRO (%)
Iso-Valeric acid	37.69
3-methyl valericacid	17.24
Seychellene	6.49
7-Tetracycle undecanol, tetramethyl	2.55
B-Patchoulane	1.71
Patchouli alcohol	3.00
¥-Gurjunene	3.40
B-Selinene	1.00
Guaiene	1.02
Aromadendrene	1.62
Calarene	3.24
Caryophyllene	1.03

Valeriana wallichii DC belonging to family Valerianaceae is popularly known as Indian Valerian (English), Mushkibala (Kashmiri), Sughanthdawal or Tagar in Sanskrit. The plant has been valued for Ayurveda in Indian, Unani in ancient Greek and Burma. It grows at the altitude ranging from 1500 to 2600 m. In Nepal, it is found in central and western hills. This plant is found growing from 1000 to 2500 m altitude in cold and shady places.

V. wallichii is a tufted, hairy herbaceous perennial, gynodioecious herb with hermaphrodite plants ranging from 13.00 – 37.70 cm in height and female plants found usually dwarf than hermaphrodite with height from 10.90- 29.50cm. The plant is characterized by thick horizontal roots with diameter ranging from 2.00 – 4.40 cm with roots ranging from 10 – 37 per stock. Basal radical leaves are long stalked, deeply cordate-ovate, usually toothed or sinuate up to 3.20 – 8.30 cm long and 2.40 – 7.50 cm broad. Cauline leaves are only a few, much smaller, entire or sometimes pinnate of 1.90-2.70 cm in length and 1.60 – 2.30 cm in breadth. Flowers are white or tinged with pink in terminal corymbs with 8 – 13 female flowers per inflorescence and 8 -14 hermaphrodite flowers per inflorescence which are larger and broader than female flowers and are ranging from 0.30 – 0.40 cm across. The seeds are planted in the nursery in February-March. The nursery soil should be irrigated to keep it moist. Roots cuttings do vegetative propagation of this herb. The plant is easily propagated from a portion of the old rootstock either in autumn or in spring.

*Cyperus rotundus***Chemical examination of CRRO**

The classical method of hydrodistillation using a Clevenger type apparatus for 4 hours was used for the isolation of the essential oil from roots of *C. rotundus* (1.0 kf).

The essential oil CRRO was collected (Yield: 0.70 %) and stored at 40 C until further use.

Table 3: Chemical constituents and retention indices of CRRO.^[15]

Chemical constituent	CRRO
A-Pinene	1.33
B-Pinene	1.75
A-Copaene	6.60
Ledene oxide	2.68
8-oxo-9H-Cycloisolongifolene	1.58
Cyperene	22.66
¥-Gurjunene	3.93
§- Cadinene	2.44
Azulon	1.54
α- Selinene	3.02
β-Selinene	6.10
Alloaromadendrene	1.01
Guaiene	1.19
Trans-Caryophyllene	1.74
Aromadendrene	1.37
Caryophyllene oxide	5.15
Valencene	2.44
Germacrene B	2.83
α- Cyperone	12.80
Caryophylla-4(12),8(13)-dien-5β-o1	1.51

The roots of *Cyperus rotundus* L., a perennial herb, of the family Cyperaceae, grown in tropical areas and along roadsides, sandy field and cultivated ground in such countries as the Bahamas, Java, Samoa, China, Egypt, Sudan, Turkey, Iran, India, France and Venezuela.

Cyperus rotundus plant is ranked as the oldest cultivated plant of ancient Egypt. It is native to warm temperate to sub-tropical regions of the Northern Hemisphere and is indigenous to western Asia and Africa, recorded as occurring scattered from Punjab to the Nilgiri hills. It is a roots crop, which grows widely in wet places as a grass and is sometimes cultivated for its small and sweet edible tubers. The plant grows to about 0.6 m by 1m, the scented flowers are hermaphrodite (have both male and female organs).

The plant cannot grow in the shade and it requires moist or wet soil. It is an erect, perennial, grass like sedge, single-stemmed, erect, graminoid, up to 3 ft. tall underground. Along with fibrous roots, there are many slender roots which form a tuber at each end. The leaves become grass-like and the blades are light green, smooth, glossy, and glabrous in texture. The plant has an extensive and complex system of fine, fibrous roots with small hard, spherical tubers and basal bulbs (swelled roots tips which produce stems and leaves) attached.

Polianthes tuberosa

Chemical examination of PTC

Chemical characterization of *P. tuberosa* concrete PTC has been carried out using GC-MS technique and chemical constituent of PTC are listed in Table 4.

Table 4: Chemical constituents of PTC.

Chemical constituent	PTC (%)
Dehydronerol isovalerate	0.01
Phenol	--
1,8 – Cineole	0.46
Benzyl alcohol	0.01
Methyl benzoate	0.79
Methyl caprylate	--
Phenylethyl alcohol	0.17
Benzyl nitrile	0.22
α – Terpineol	--
Benzoic acid	--
Benzyl acetate	0.13
Ethyl benzoate	--
Indole	0.02
Dimethyl salicylate	0.05
Methyl salicylate	1.45
Methyl vanillin	--
Methyleugenol	0.36
Methyl anthranilate	0.21
Methyl isoeugenol	8.47
Benzyl benzoate	1.01
1,19-Eicosadiene	3.89
Eicosane	1.09
9,12,15- Octadecatrien-1-ol	3.52
8,11-Octadecadienoic acid, methyl ester	3.95
10-Heneicosene(C.T)	10.59
1-Eicosanol	1.64
Hexadecanoic acid, cyclohexy ester	1.00
Pentacosane	8.36
Heptacosane	12.86

2,3-Bis[(trimethylsilyl) oxy] propyl-(9z,12z)-9,12-octadecadienoate	1.80
Nonacosane	19.46
1-[3-(Octadecyloxy)propoxy] octadecane	2.40

Flowering plants are valued much by the aesthetic world for the beauty and fragrance of their flowers. Among the flowering plants, *Polianthes tuberosa* occupies a very special position because of its prettiness, elegance and sweet pleasant fragrance. This bulbous plant is the source of *Polianthes tuberosa* oil of commerce which is very expensive and used in high grade perfumery. *P. tuberosa* is also cultivated for cut flowers and for preparing bouquets and garlands. The long flower spikes of *P. Tuberosa* are excellent as cut flowers for table decoration. The individual florets are used for making garlands, floral ornaments, bouquets and button-holes.

Polianthes tuberosa L. is believed to have originated in Mexico or Central America. It is widely cultivated in Southern France and also in Morocco for the Extraction of its natural flower oil. For many years *P. tuberosa* flower oil has been one of the most valuable and expensive perfumery raw materials. But later, it has recorded a declining trend in production and utilization. *Polianthes tuberosa L.* belongs to the family Asparagaceae. There are single as well as double flowered varieties. Single flowered type is mostly cultivated for the extraction of its perfume while the double flowered variety usually goes to the cut flower trade. The flowers on top of the long stalk are grouped in spike-shaped clusters 15 – 20 cm long. There are four groups of cultivars of tuberose. (i) Single: Most widely cultivated, flowers are pure white and have got a single row of corolla segments. Eg. 'Calcutta single', tinged with pinkish red. Petals are in several whorls. Eg. 'Pearl' and 'Calcutta double'. (iii) Semi-double: Similar to double but with only 2 -3- rows of corolla segments. (iv) Variegated: Variegated leaves with yellow margins.

Chemicals Used

1-Diphenyl 1-2-picrylhydrazyl (DPPH) radical, ascorbic acid, potassium ferricyanide, trichloroacetic acid (TCA), hydrogen peroxide, phosphate buffer (pH 7.4), methanol, ethanol, hexane, CCl₄, dextrose, sodium citrate, citric acid, NaCl, distilled Water, CO₂, α -tocopherol, Tris-HCL buffer, nitroblue tetrazolium chloride (NBT), Butylated hydroxyl anisole (BHA), ethylene tetra ammine acetic acid (EDTA), thiobarbituric acid (TBA), nicotinamide adenine dinucleotide (NADH), deoxyribose, hyposaline, diclofenac, tetracycline, carbendazim and ferric chloride. All chemicals including solvents used were of analytical grade.

Pharmacological study

Anti-inflammatory assay

Experimental Methodology

The antioxidant activity of hydrodistilled oils was analysed by the methods of HRBC radical scavenging activity.

Human Red Blood Cell Membrane Stabilization (HRBC) method

In the human red blood cell membrane stabilization method used for this study, blood collected from healthy human volunteers who had not taken non steroidal antiinflammatory drugs (NSAIDs) for 2 weeks prior to the experiment was mixed with equal volume of Alsever solution (2.0 % dextrose, 0.80%, sodium citrate, 0.50 %, citric acid and 0.42% sodium chloride) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made.

Various concentrations of extracts were prepared using distilled water and to each concentration, 1.0 ml of phosphate buffer, 2.0ml of hyposaline and 0.50 ml of HRBC suspension were added and the mixture was incubated at 37⁰c for 30 min and centrifuged at 3,000 rpm for 2 min; the haemoglobin content of supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac was used as the standard and a control was prepared omitting the test drugs. The percentage of HRBC membrane stabilization was calculated using the formula,

$$\% \text{ Inhibition} = [(ABS_{\text{control}} - Abs_{\text{sample}})] * 100$$

where, ABS_{control} is the absorbance of the control and Abs_{sample} is the absorbance of sample/standard.

Antimicrobial activity assay

Experimental Methodology

Agar well-diffusion method for antimicrobial studies

The essential oils were tested for antibacterial activity by agar well-diffusion method against pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus* species, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The pure cultures of bacteria were grown in Nutrient broth for 25 hours. Approximately 20 ml of molten and cooled Mueller Hinton agar was poured in sterilized petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 6mm diameter were made on Mueller Hinton agar

(MHA) plates using sterilized stainless steel cork borer. Using a micropipette, different concentrations of the sample (5.0 µg/ml – 40.0 µg/ml) were poured onto each well on all plates. Test results were compared for tetracycline (positive control) and universal solvent water (negative control). The plates were incubated for 24 hours at 37⁰c and after incubation, the different levels of zone of inhibition of bacteria were measured using a meter ruler and the mean value for each organism was recorded and expressed in millimetre.^[12]

Antioxidant assay

Experimental Methodology

DPPH radical scavenging activity

Various concentrations of hydrodistilled oils and the extracts of CRRO were mixed separately with 2.7 ml of methanol solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and allowed to stand for 60 min in dark. The reduction of the DPPH radical was determined by reading the absorbance at 517 nm.

Ascorbic acid and α -tocopherol were used as standards. The Radical Scavenging Activity (RSA) was calculated as a percentage of DPPH discolouration, using the formula;
 $\%RSA = [(Abs\ control - Abs\ sample) / (Abs\ control)] \times 100$ where, Abs control is the absorbance of the control and Abs sample is the absorbance of sample/standard.^[13]

CNS depressant activity of PTC by in vivo method

Mental diseases including anxiety and depression are considered to be a leading global healthcare burden. This amounts to 12.3 % of the global burden of disease and is expected to rise further in the near future. Approximately 450 million people suffer from a mental or behavioural disorder, but only a small portion of them receive even the most basic treatment. Moreover, most of the conventional drugs available for the treatment of mental and behavioural disorders possess serious risks of adverse effects.

Medicinal herbs have been used as a form of therapy in pain management throughout history. These plants have also proven to be an important source of new chemical substances with prospective therapeutic effects. It is therefore not amazing that most of the important analgesic prototypes (e.g. Salicylic acid and Morphine) were originally derived from plant sources. The treatment of chronic pain is an area in which the practitioners of traditional medicine enjoy patronage and success.

Among the flowering plants which are valued much by the aesthetic world for beauty and fragrance, *P. tuberosa* occupies a very special position because of its prettiness, elegance and sweet pleasant fragrance.

EXPERIMENTAL METHODOLOGY

Pentobarbitone Induced Sleeping Time Test

The animals were randomly divided into five groups consisting of six mice each. The test groups received PTC at doses of 100,200 and 400 mg/kg respectively while positive control was treated with diazepam (1 mg/kg i.p.) and control with vehicle (1 % Tween 80 in water). Thirty minutes later, pentobarbitone (40 mg/kg, i.p., Sigma Chemicals, USA) was administered to the animals in each mouse to induce sleep. The animals were observed for the latent period (time between pentobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex).

RESULT AND DISCUSSION

The aroma plants commonly found in nature which has medicinal values. These plants are used in auryeudic medicine and have been mentioned in ancient texts for various medicinal activities such as anti inflammatory, anti microbial, anti oxidant activities, which are given in Table 5.

Table 5: The anti-oxidant, anti-inflammatory, anti-microbial activity and central nervous system of aroma plants.

	Antioxdant Activity	Antiinflammatory Activity (Inhibition %)	Antimicrobial Activity	CNS Activity
<i>Valeriana Wallichii (Root)</i>	Yes	Yes 69%	Yes	No
<i>Cyperus Rotundus (Rhizomes)</i>	Yes	Yes 56%	Yes	No
<i>Polianthes Tuberosa</i>	Yes	No	Yes	Yes 74

Antimicrobial activity of VWRO

The result of the antimicrobial activity of the tested plant extracts in methanol are summarized in (fig 1). The highest zone of Inhibition (zoi =13 mm) was observed against Bacillus species, followed by *S. aureus* has (zoi=12mm). Significant inhibition was also observed against *E.coli* and *K.pneumoniae* (zoi= 11 mm) followed by *P. aeruginosa* with least inhibition (zoi= 9mm).

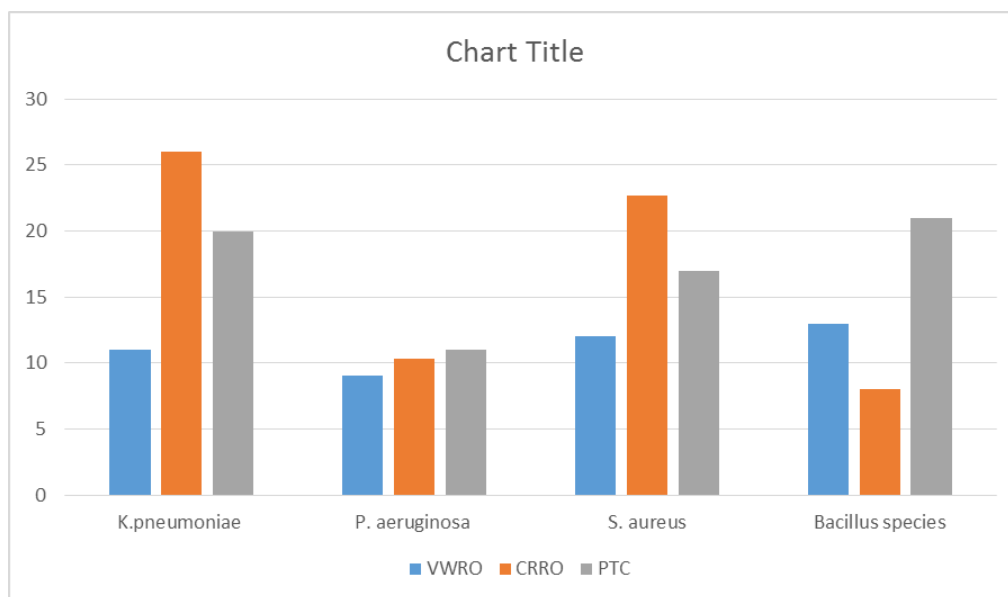


Figure 1: The Anti-microbial activity of aroma plants.

Anti-microbial activity of CRRO

The antimicrobial activity of the tested plant extracts in methanol are summarized in Table 5. The highest zone of Inhibition (zoi =26.0mm) was observed against K.pneumoniae, followed by S. aureus has (zoi=22.7mm). Significant inhibition was also observed against E.coli and P. aeruginosa (zoi= 10.3 mm) followed by Bacillus species with least inhibition (zoi= 8.0mm).

Anti-microbial activity of PTC

The antimicrobial activity of the tested plant extracts in methanol are summarized in Table 5. The highest zone of Inhibition (zoi =21.0mm) was observed against Bacillus species, followed by K.pneumoniae has (zoi=20.7mm). Significant inhibition was also observed against E.coli and S. aureus (zoi= 17. mm) followed by P. aeruginosa with least inhibition (zoi= 11.0mm).

Anti-oxidant activity of VWRO

VWRO extract showed an increase in total reducing power from absorbance of 0.086 to 0.213 in the concentration of 0.25mg/ml to 2.0mg/ml at wavelength of 517nm.

Anti-oxidant activity of CRRO

CRRO extract showed an increase in total reducing power from absorbance of 0.18 to 0.78 in the concentration of 0.2mg/ml to 1.0mg/ml at wavelength of 517nm.

Anti-oxidant activity of PTC

PTC extract showed an increase in total reducing power from absorbance of 0.6 to 2.3 in the concentration of 0.2mg/ml to 1.0mg/ml at wavelength of 517nm.

By this study we can understand that a large number of biologically active phytochemical has diverse variety of pharmacological properties, as described above, has been found in the treatment of a various diseases. Its therapeutic effects are excellent and no adverse reaction was observed.

CONCLUSION

The above collected information suggest that *valeriana wallichii*, *Cypreus rotundus* and *polyanther tuberosa* has some important pharmacology properties traditional uses of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use. Ayurvedic and traditional practitioners for treatment of ailments. Researchers are exploring the therapeutic potential of these plants as it has more therapeutic properties which are not known.

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