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Research Article

IN VITRO FREE RADICAL SCAVENGING ACTIVITY AND PHENOL AND FLAVONOID CONTENT OF *NERIUM INDICUM*, *PELTO PHORAMPTEROCARPUM* AND *ROSA* SPPS. FLOWER EXTRACTS

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ABSTRACT

Objective: The aim of the present study was to evaluate antioxidant activity and phenol and flavonoid content of three flowers viz. *Nerium indicum*, *Peltophoram pterocarpum* and *Rosa spps* extracts.

Methods: The extraction was done by individual cold percolation method using solvents like hexane, ethyl acetate and acetone. Total phenolic and total flavonoid content of different extracts was determined using Folin-ciocalteu assays and aluminium chloride colorimetric method respectively. Antioxidant activity was carried out by ferric reducing antioxidant power (FRAP) and ABTS radical cation scavenging activity.

Results: Total phenol content was more in acetone extract while total flavonoid content was more in ethyl acetate extract, in all the three flower species. FRAP activity was maximum in acetone extracts while ABTS activity was more in ethyl acetate extracts.

Conclusion: Phenolic and non-phenolic compounds contribute to the antioxidant activity of these three flower extracts. The best antioxidant activity was shown by *P. pterocarpum* flowers suggesting it to be a good source of natural antioxidants.

Keywords: Flowers, Antioxidant activity, 2, 2'-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid, Ferric reducing antioxidant power, Total phenol content, Total flavonoid content.

INTRODUCTION

The plants have been source of medicinal agents for thousands of years and a number of novel and modern drugs have been isolated from them. Various phytochemicals found in plants have a beneficial effect on health or play an active role against various diseases [1]. Medicinal plants, during development produce a variety of secondary metabolites in which phenolic compounds play a key role as antioxidants [2]. Free radicals are capable of existing with one or more unpaired outer shell electrons, extremely reactive and generally highly unstable [3]. They cause oxidation of biomolecules leading to cellular damage, DNA damage, cell functions inhibition and are mediators of many diseases and also play a major pathogenic role in the aging process [4,5]. Synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene are widespread food additives; however, their use is increasingly prohibited, due to their potential health risks and toxicity [6]. In recent years, there has been growing interest in finding natural antioxidants in plants because they show strong protection against the damages of the cellular organelles caused by free radical induced oxidative stress, inhibit oxidative damage and may prevent aging and neurodegenerative diseases [7,8]. The antioxidant activity of natural compounds is mainly due to their redox properties [9]. The natural antioxidants function as free-radical scavengers and chain breakers, complexes of pro-oxidant metal ions and quenchers of singlet-oxygen formation [10]. All living organisms have endogenous defense systems against oxidative damage. There are two main antioxidant defense mechanisms. The first one is antioxidant defense with enzymes like superoxide dismutase and catalase, and the second one is non- enzymatic such as polyphenols, flavonoids, ascorbic acid, phenolic acids, tannins phenolic diterpenes and carotenoids [11].

Phenolic compounds, ubiquitous in plants are an essential part of the human diet and are of considerable interest due to their antioxidant properties. Phenolic compounds also exhibit a wide range of pharmacological properties, such as anti-allergenic, anti- atherogenic, antiinflammatory, anti-microbial, anti-thrombotic, cardioprotective, and vasodilatory effects [12]. Any part or rather all plant parts are capable of showing antioxidant properties. In general, leaves are more often evaluated for their antioxidant properties, but it was thought of interest to evaluate flowers for their antioxidant ability. Flowers are generally used for religious purpose and thrown away into the environment. It will be dual benefit if they are used therapeutically. Hence in the present work, three flowers *viz. Nerium indicum, Peltophoram pterocarpum* and *Rosa spps.* were evaluated for antioxidant efficacy. Total phenolic and total flavonoid contents were also measured.

Plant description

The description of the flowers and the therapeutic uses are given below.

N. indicum L. belongs to the family Apocynaceae. It is commonly found throughout India. The vernacular name is Karen. *N. indicum* is used

in the treatment of asthma, cardiac illness and diabetes mellitus. The flowers and leaves are used to stimulate cardiac muscles, relieve pain, and eliminate blood stasis. It is reported to have antibacterial and antidiabetic activities. Antioxidant activities have been reported by Vinayagam and Sudha [13].

P. pterocarpum DC. belongs to the family Fabaceae. It is a small to medium-size tree, 5-10 m tall, commonly found throughout India and Sri Lanka. The vernacular name is *Peltophorum*. The tree is widely grown in tropical regions as an ornamental flower. Young leaves and pods are eaten by livestock. The timber is used for furniture. In traditional medicine, *P. pterocarpum* flowers are used as an astringent to cure or relieve intestinal disorders after pain at childbirth, sprains, bruises and swelling or as a lotion for eye troubles, muscular pains and sores [14]. Some of the reported activities are cardiotonic, antioxidant, antiglycemic and antimicrobial activity [15-17].

Rosa spp. belongs to the family Rosaceae. It is a deciduous shrub growing up to 2.2 m. It is commonly found throughout India. The vernacular name is gulab. The flowers are commonly used in traditional Chinese medicine. The flowers are reported to have astringent, analgesic, anti- inflammatory, antidepressant, antibacterial, antioxidant, diuretic, and anti-HIV activity [14,18-19].

METHODS

Plant collection

The flowers of *N. indicum, P. pterocarpum and Rosa spps.* were collected in the month of September 2013 from the campus of Saurashtra University, Rajkot, Gujarat, India. The flowers were washed thoroughly with tap water, shade dried and homogenized to a fine powder and stored in air tight bottles.

Extraction method

The dry powder of three different flowers was individually extracted by cold percolation method using different organic solvents like hexane (HE), ethyl acetate (EA) and acetone (AC) [20]. 10 g of dried powder was taken in 100 ml HE in a conical flask, plugged with cotton wool and then kept on shaker at 120 rpm for 24 hrs, the extract was filtrated with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 minutes. The supernatant was collected, and the solvent was evaporated. The residue was then taken in 100 ml of solvents (EA and AC) in a conical flask, plugged with cotton wool and then kept on a shaker at 120 rpm for 24 hrs. Then the procedure followed same as above, and the dry extract was stored in air tight bottles. The extract was weighed, and the extractive yield was calculated.

Determination of total phenol content

The amount of total phenol content of EA and AC extracts of three different flowers were determined by Folin–Ciocalteu's reagent method [21]. The extract (0.5 ml) and 0.1 ml of Folin–Ciocalteu's reagent (0.5 N) were mixed and the mixture was incubated at room temperature for 15 minutes. Then, 2.5 ml of sodium carbonate solution (2 M) was added and further incubated for 30 minutes at room temperature and the absorbance was measured at 760 nm using a digital spectrophotometer (Systronics, India), against a blank sample. The calibration curve was made by preparing gallic acid (10-100 µg/ ml) solution in distilled water. Total phenol content is expressed in terms of gallic acid equivalent (mg/g of extracted compounds).

Determination of flavonoid content

The amount of flavonoid content of EA and AC extracts of three different flowers was determined by aluminum chloride colorimetric method [22]. The reaction mixture (3.0 ml) consisted of 1.0 ml of sample (1 mg/ml), 1.0 ml methanol, 0.5 ml of aluminum chloride (1.2%) and 0.5 ml potassium acetate (120 mM) and was incubated at room temperature for 30 minutes. The absorbance of all samples was measured at 415 nm using a digital spectrophotometer (Systronics, India), against a blank sample. The calibration curve was made by preparing a quercetin (5-60 µg/ml) solution in methanol. The flavonoid content is expressed in terms of standard equivalent (mg/g of extracted compound).

Antioxidant activity

The antioxidant activity of EA and AC extracts of three different flowers were evaluated by ferric reducing antioxidant power (FRAP) and 2, 2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity.

FRAP

The reducing ability of EA and AC extracts of three different flowers was determined by FRAP assay [23]. FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tripyridyl- s-triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH- dependent (optimum pH 3.6). 0.1 ml extract is added to 3.0 ml FRAP reagent (10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ in 40 mM HCl and 1 part 20 mM $FeCl_3$) and the reaction mixture is incubated at 37°C for 10 minutes and then the absorbance was measured at 593 nm. $FeSO_4$ (100-1000 µM/ml) was used as a positive control [24]. The antioxidant capacity is based on the ability to reduce ferric ions of the sample was calculated from the linear calibration curve and expressed as M $FeSO_4$ equivalents per gram of extracted compound.

Determination of ABTS radical cation scavenging activity.

The ABTS radical cation scavenging activity of EA and AC extracts of three different flowers was determined by the method described by Re *et al.* [25]. ABTS radical cations are produced by reaction of ABTS (7 mM) with potassium persulfate (2.45 mM) and incubating the mixture at room temperature in the dark for 16 hrs. The working solution obtained was further diluted with methanol to give an absorbance of 0.85±0.20.

1.0 ml of different concentrations of solvent extracts and fractions of the seven flowers diluted by methanol was added to 3.0 ml of ABTS working solution. The reaction mixture was incubated at room temperature for 4 minutes and then the absorbance was measured at 734 nm using digital spectrophotometer (Systronics, India), against a blank sample. Ascorbic acid (1-10 µg/ml) was used as a positive control [26]. Percentage of inhibition was calculated as described earlier.

RESULTS AND DISCUSSION

The extractive yield varied with different solvents in different flower extracts. In non-polar solvent HE, maximum extractive yield was in *P. pterocarpum* and minimum in *Rosa* spps. In semi-polar solvent EA, the extractive yield was almost same in all the three flower extracts. In polar solvent AC, maximum extractive yield was in *P. pterocarpum* and minimum in *Rosa* spps. (Fig. 1). It is a well-known fact that the extractive yield is greatly dependent on the extraction solvent and it varies from plant to plant and solvent to solvent and part of the plant being extracted [27,28]. The extractive yield is influenced by the polarity of the solvent used for extraction and the solubility of particular phytoconsituent in the solvent used for extraction [29,30].

The total phenol content varied with different solvents in different flower extracts. On the whole, the total phenol content was more in AC extract than in EA extract irrespective of the flower extract evaluated. In semi-polar solvent EA extract, maximum total phenol content was in *Rosa* spps and minimum in *N. indicum*, while in polar solvent AC extract, maximum total phenol content was in *Rosa spps.* and minimum in *N. indicum* (Fig. 2). The total flavonoid content also varied with different solvents in different flower extracts. On the whole, the total flavonoid content was more in EA extract than in AC extract irrespective of the flower extract evaluated. In semi polar solvent EA extract, maximum total flavonoid content was in *Rosa spps* and minimum in *P. pterocarpum* while in polar solvent AC extract, maximum total flavonoid content was in *Rosa spps* and minimum in *P. pterocarpum* (Fig. 3). The polarity of the solvents affect the extraction efficiency of phenolic compounds from the plant materials [31]. The chemical characteristic nature of the solvent and the diverse structure and composition of the natural products are responsible for differences observed in the phenolic content of the different flowers extracts [32].

The ability of the extracts to reduce ferric ion to ferrous ion was determined and the result was presented as $FeSO_4$ equivalent. The FRAP varied with different solvents in different flower extracts. On the whole, the FRAP activity was more in AC extract than in EA extract irrespective of the flower extract evaluated. FRAP activity of *P. pterocarpum* and *Rosa spps* was same in semi polar solvent EA extract while it was considerably less in *N. indicum*. In polar solvent AC extract, maximum FRAP was in *P. pterocarpum* and minimum in *N. indicum* (Fig. 4). Molehin and Adefegha also reported maximum FRAP activity in polar solvent ethanol in *Momordica foetida* plant [33]. Since FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidant present, it can be stated that AC extract of *P. pterocarpum* is capable of converting free radicals into stable non radical species. The observed antioxidant property can be attributed to its phenolic content.

Proton radical scavenging is an important characteristic feature of antioxidants. ABTS a protonated radical has characteristic absorbance maxima at 734 nm that decreases with the scavenging of the proton radicals [34]. This antioxidant assay is very appropriate and is often used to screen the antioxidant capacity of plant extracts because of its ability to be used both organic and aqueous media and its stability in a wide pH range [35]. The ABTS radical cation scavenging activity of different solvent extracts in different flower extracts is given in Figs. 5 and 6. The activity was different in different flower extracts and also different in different solvents. The range of inhibitory concentration 50 (IC_{50}) values was from 54 ug/ml to 228 ug/ml. Lowest IC_{50} value was in EA extract of *P. pterocarpum* (54 µg/ml) followed by EA extract of *Rosa spps* (90 µg/ml). AC extract of *P. pterocarpum* and *N. indicum*

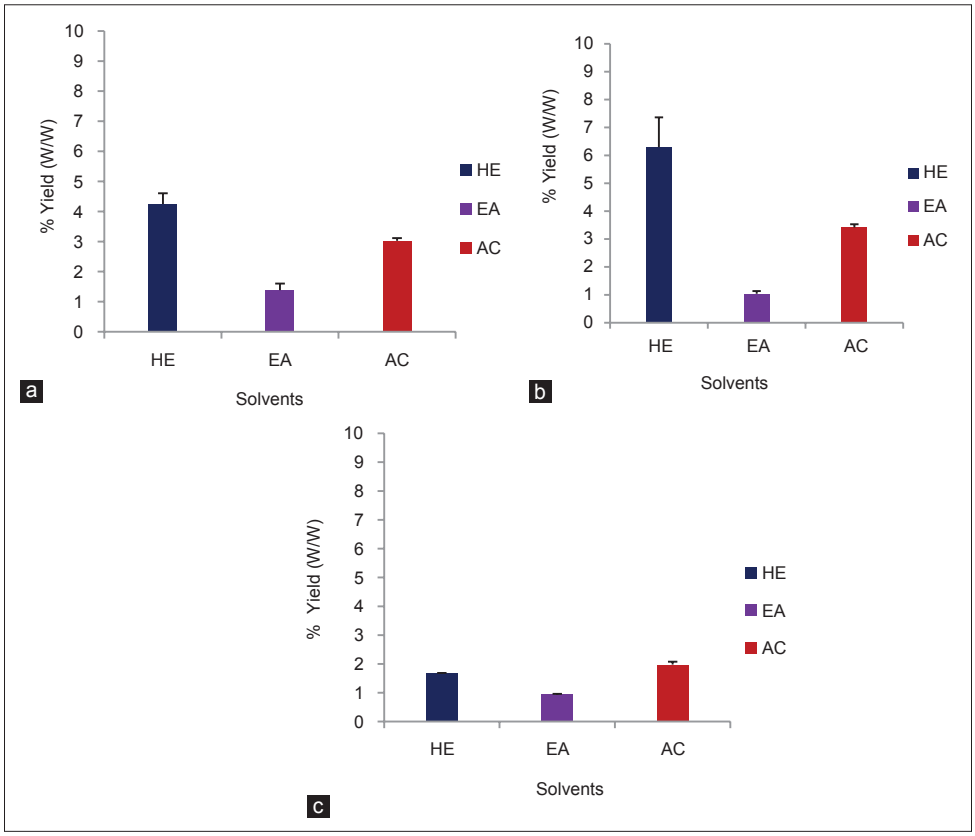

Fig. 1: Extractive yield of different solvents extracts of different flower as: (a) *Nerium indicum*, (b) *Peltophoram pterocarpum*, (c) *Rosa spps*

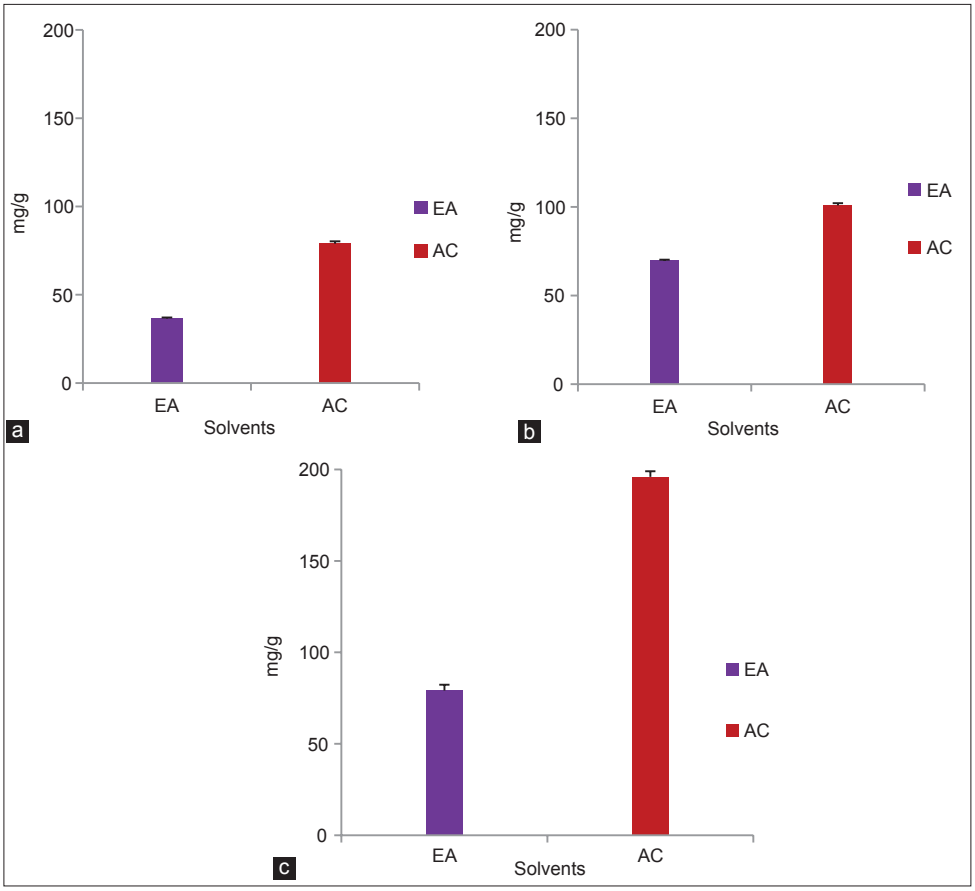

Fig. 2: Total phenol content of different solvent extracts of different flower as: (a) *Nerium indicum*, (b) *Peltophoram pterocarpum*, (c) *Rosa spps*.

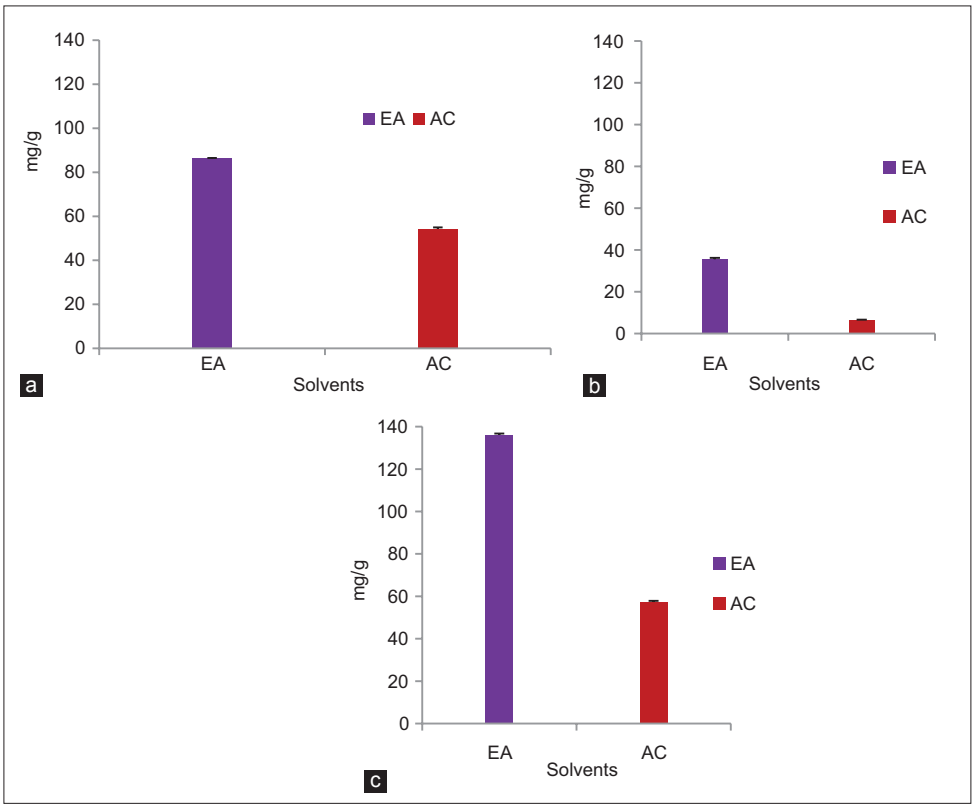

Fig. 3: Total flavanoid content of different solvent extracts of different flower as: (a) *Nerium indicum*, (b) *Peltophoram pterocarpum*, (c) *Rosa spps*.

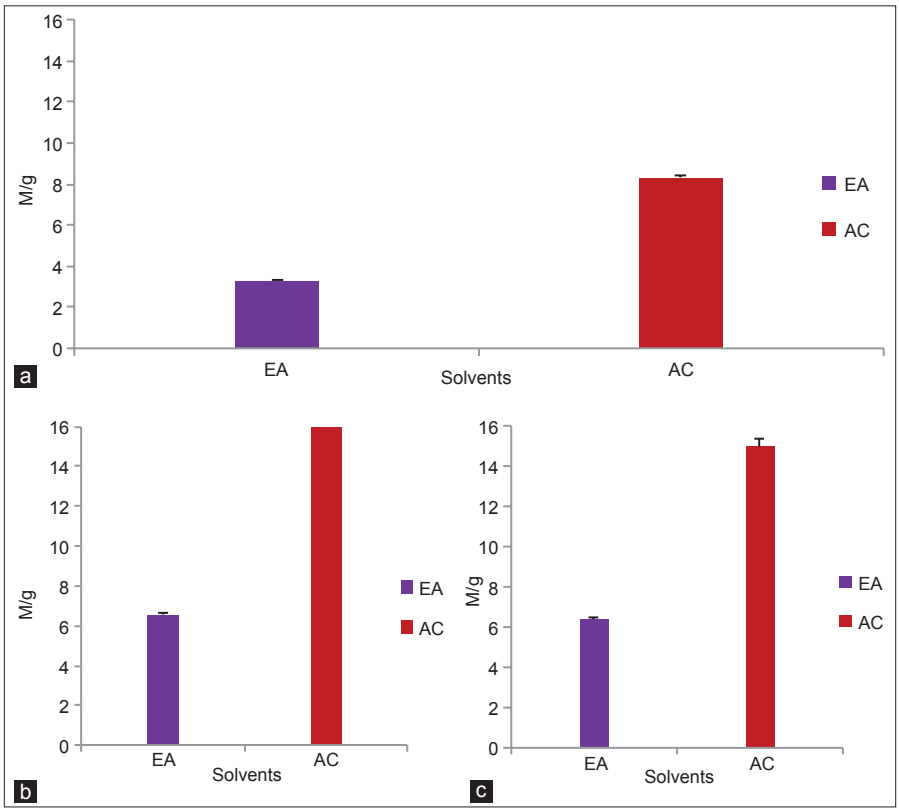

Fig. 4: Ferric reducing antioxidant power of different solvent extracts of different flower as: (a) *Nerium indicum*, (b) *Peltophoram pterocarpum*, (C) *Rosa spps.*

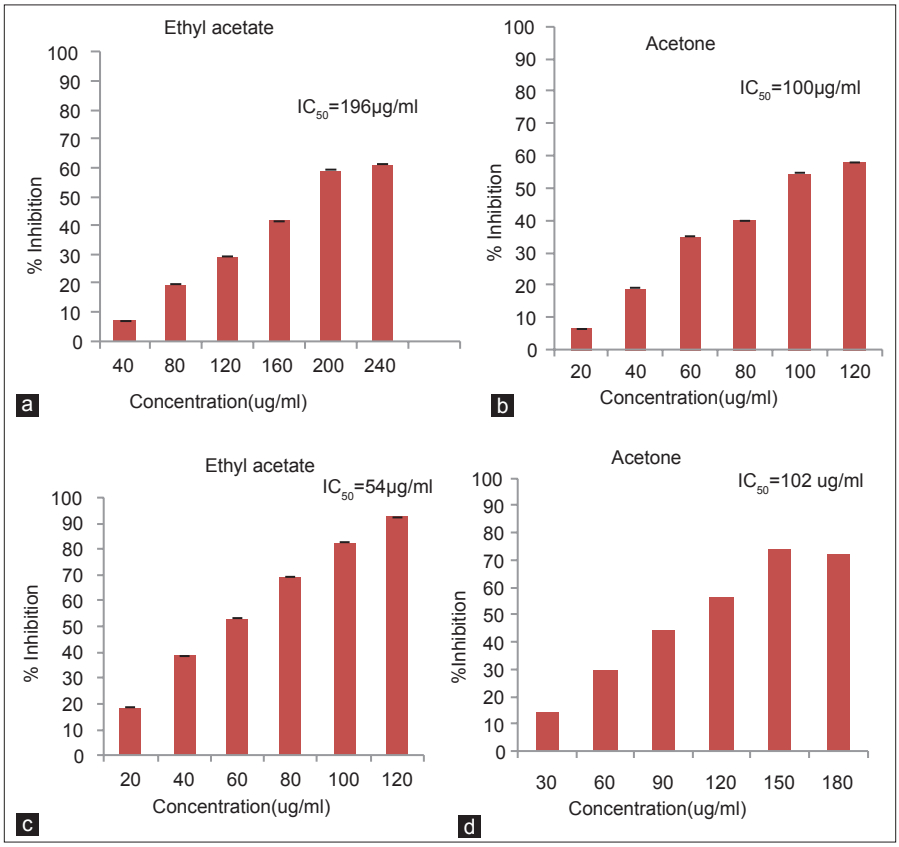

Fig. 5: 2, 2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid activity of different solvents extracts of diffrent flower as: (a and b) *Nerium indicum*, (c and d) *Peltophora pterocarpum*

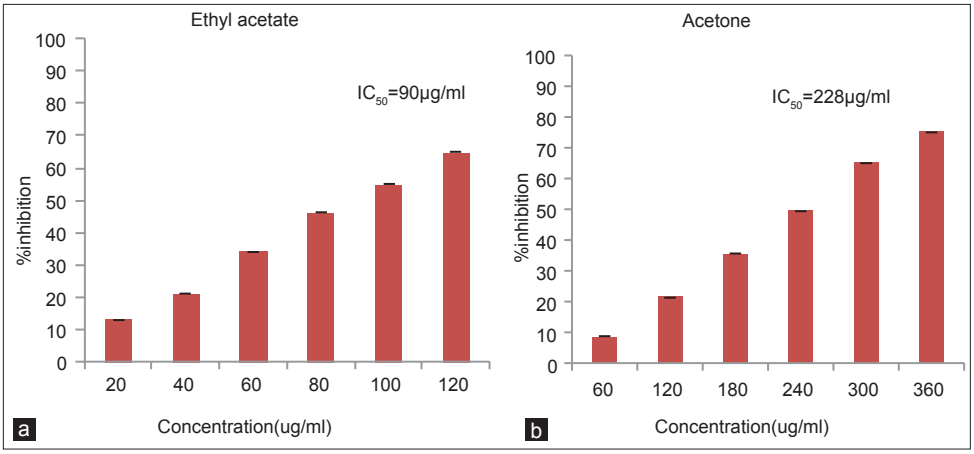

Fig. 6: 2, 2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid activity of different solvents extracts: (a and b) *Rosa spps*.

had almost same IC_{50} value (102 µg/ml). *P. pterocarpum* and *Rosa spps* showed direct correlation with total flavonid content i.e. the EA extracts of both these flower extracts had more flavonoid content and had high ABTS antioxidant activity as evidence by lower IC_{50} values. Sudha *et al.*, also reported high ABTS radical scavenging activity by EA extracts of *Solanum muricatum* fruit [36].

In the present study, EA and acetone extracts of three flower species were evaluated for their antioxidant efficacy. It is well known that any one single method in not sufficient for evaluation of antioxidant activities of different plants because of their complex composition [37]. Therefore, it is suggested that at least two or more antioxidant assays must be evaluated to get relevant data; hence in the present study antioxidant activity of flower extracts were measured by two antioxidant assay *viz.* FRAP and ABTS. In both the assays, there is a single electron transfer reaction [38]. The EA and AC extracts of *P. pterocarpum* showed good antioxidant activity. Hence it can be used as a source of natural antioxidants and may be useful for curing diseases caused by oxidative stress.

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