World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Evaluation of antioxidant potential and phenol and flavonoid content of some flower extracts of Saurashtra region

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Received: 20-05-2014 / Revised: 06-06-2014 / Accepted: 18-06-2014

ABSTRACT

To investigate antioxidant efficacy of ethyl acetate and acetone fractions of four flowers viz. *Alstonia scholaris, Calotropis procera, Cassia auriculata* and *Catharanthus roseas* and estimation of total phenol and flavonoid content. Extraction was done by cold percolation method using hexane, ethyl acetate and acetone solvents. Different fractions of four flowers were tested for total phenol and flavonoid content. *In vitro* antioxidant activity was measured by two antioxidant assays viz. ABTS assay and FRAP activity. *C. auriculata* had maximum phenol content and showed maximum FRAP and ABTS activity while *C. procera* had lower phenol content and lower FRAP and ABTS activity indicating a positive correlation between phenolic content and antioxidant activity. *C. auriculata* was best among the four flowers screened and can be considered as a promising source for the treatment of many free radical related diseases.

Key words: *Alstonia scholaris, Calotropis procera, Cassia auriculata, Catharanthus roseas*, FRAP, ABTS, flower extracts, total phenol content

INTRODUCTION

Medicinal plants are traditionally used for the treatment of various diseases in India and all over the world since the beginning of civilization. In fact, natural products are a source of synthetic and traditional herbal medicine. They are the primary health care system in some parts of the world [1]. Medicinal plants are a source of great economic value in the Indian subcontinent. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times.

Medicinal plants have been playing a vital role on the health and healing of man since dawn of human civilization. There is a cure of every disease or disorder in nature and traditionally the plants are being used to treat them. The medicinal plants are reported to possess various pharmacological activities like anti-inflammatory[2,3], hepatoprotective[4], antibacterial[5,6], antioxidant[7,8], anticancer[9], antiulcer [10,11], antiurolithiatic[12], antiepileptic[13], etc. All plant parts are potential source of medicinal substances. All plant parts like seed, leaf, stem, bark, flower, peel, fruit rind, aerial parts, etc are known to possess medicinal properties and show different pharmacological activities. For eg. antibacterial and antiinflammatory activity of *Woodfordia fruticosa* flowers[14,15], antibacterial and antioxidant activities of *Schotia latifolia* stem bark[16], Antibacterial, antioxidant activities of *Melastoma malabathricum* leaves [17], anti ulcer and antibacterial activity of *Polyalthia longifolia* leaves[18] , *in vitro* anti-candida activity of *Heracleum persicium* fruit[19], i*n vitro* antibacterial activity of *Emblica officinalis* and *Tamarindus indica* seed[20], etc.

Free radicals (reactive oxygen and nitrogen species, ROS/RNS) are produced in normal and pathological cell metabolism and they are controlled by endogenous enzymes such as superoxide dismutase, glutathione peroxidase, catalase or chemical compounds such as a-

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tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione. The large production of free radicals results in the onset of many diseases [21]. Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Antioxidants are involved in the defense mechanism of the organism against the pathologies associated with the attack of free radicals [22]. Antioxidants are vital substances because they can protect the body from the damage caused by free radicals. Many synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) are very effective. However, they possess certain side effects and are toxic to humans [23]. The synthetic antioxidants have restriction for use, as they are suspected to be carcinogenic. Therefore, the need for searching and exploiting natural antioxidants has increased greatly. Medicinal plants are source of certain bioactive molecules which act as antioxidants [24]. They can protect the human body against both cellular oxidation reactions. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke [25]. Thus it is important to characterize different types of medicinal plants for their antioxidant potential [26].

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. The antioxidant activities of phenolics are related to a number of different mechanisms, such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl. A direct relationship has been found between the content of total phenolics and antioxidant capacity of plants [27]. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. They are widely distributed as secondary metabolites in the plant kingdom. Flavonoids in biological systems are ascribed to their antioxidant abilities, capacity to transfer electrons, quenching of free radicals and chelating abilities, activate antioxidant enzymes, reduce alpha tocopherol radicals and inhibit oxidases[28].

Flowers are mainly used for religious purposes after which they are thrown away into the environment. The other most important use of flowers is their use in medicine and cosmetics. There are many reports of flowers extracts showing antibacterial, antioxidant, anti cancer, hepatoprotective activities. Some of the flower extracts showing antioxidant and antibacterial activities, hepatoprotective and cytotoxicity are reported in Table 1. The flowers belonged to various families like Malvaceae, Fabaceae, Verbenaceae, Musaceae, Asteraceae, Punicaceae, Lamiaceae, etc. In the present work, four different flowers like *Alstonia scholaris, Calotropis procera, Cassia auriculata, Catharanthus roseas* were selected to evaluate their antioxidant potential.

Plant description [39]

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

Alstonia scholaris R.Br. belongs to the family Apocynaceae. The vernacular name is saptaparni. It is cultivated in India, Sri Lanka and Africa. Traditionally the flower and fruits is used in fever, malarial, leprosy, skin diseases, tumours, chronic and foul ulcers, asthma, and bronchitis.

Calotropis procera (Ait.) R.Br belongs to the family Asclepiadaceae. The vernacular name is Akado. Traditionally the root of wide variety is used in poisonous snake bite. The milky juice is used as blistering agent. Flowers are used in cholera and asthma.

***Cassia auriculata* L.** belongs to the family Ceasalpiniaceae. It is found in Sri Lanka, Indo – china, Malaya, Australia, Grain wild in South India and Sri Lanka. The vernacular name is Awal

Traditional it is used in fever, urinary diseases. The shrub is especially famous for its attractive yellow flowers which are used in the treatment of skin disorders and body odour. It is widely used in traditional medicine for rheumatism, conjunctivitis and diabetes. It has many medicinal properties. Its bark is used as an astringent, leaves and fruits anthelminthic, seeds used to treat in eye troubles and root employed in skin diseases. It is also used for the treatment of ulcers, leprosy and liver disease.

Catharanthus roseas G. Don. Belongs to the family Apocynaceae. It is commonly found throught India. The vernacular name is Barmasi. The leaves are used in diabetes. The plant is recognized to control major diseases such as leukemia and diabetes.

MATERIALS AND METHODS

Plant Collection: Four different flowers like *Alstonia scholaris, Calotropis procera, Cassia auriculata, Catharanthus roseas* were collected in the month of September 2013 from campus of Saurashtra University, Rajkot, Gujarat, India. The flowers were washed thoroughly with tap water, shade dried and crushed to fine powder and stored in air tight bottles.

Extraction Method: The dry powder of different flowers were individually extracted by cold percolation method [14] using different organic solvents like hexane (HE), acetone (AC), ethyl acetate (EA). 10g of dried powder was taken in 100 ml hexane in a conical flask, plugged with cotton wool and then kept on shaker at 120 rpm for 24h; the extract was filtrated with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 min. The supernatant was collected and the solvent was evaporated. The residue was then taken in 100 ml of solvents (ethyl acetate and acetone) in a conical flask, plugged with cotton wool and then kept on a shaker at 120 rpm for 24 h. Then the procedure followed same as above, and the dry extract was stored in air tight bottles. The extract was weighed to obtain the extractive yield.

Quantitative phytochemical analysis

Determination of total phenol content: The amount of total phenol content of ethyl acetate and acetone extracts of four flowers was determined by Folin-Ciocalteu's reagent method [40]. 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 min. Then, 2.5 ml of sodium carbonate (2 M) solution was added and further incubated for 30 min 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 to 100 µg ml^{-1}) solution in distilled water. Total phenol content is expressed in terms of Gallic acid equivalent (mg g^{-1} of extracted compounds).

Determination of flavonoid content: The amount of flavonoid content of ethyl acetate and acetone extracts of four flowers was determined by aluminium chloride colorimetric method [41]. The reaction mixture (3.0 ml) consisted of 1.0 ml of sample (1 mg ml^{-1}), 1.0 ml methanol, 0.5 ml of aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM) and was incubated at room temperature for 30 min. 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 to 60 µg ml^{-1}) solution in methanol. The flavonoid content is expressed in terms of quercetin equivalent (mg g^{-1} of extracted compound).

Antioxidant activity: The antioxidant activity of ethyl acetate and acetone extracts of four different flowers were evaluated by Ferric reducing

antioxidant power and 2,2'-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging assay.

Ferric reducing antioxidant power (FRAP): The reducing ability of ethyl acetate and acetone were determined by FRAP assay [42]. FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 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 (2,4,6- tripyridyl-s-triazine) in 40 mM HCl and 1 part 20 mM $FeCl_3$] and the reaction mixture is incubated at 37°C for 10 min and then the absorbance was measured at 593 nm. $FeSO_4$ (100 to 1000 µM ml^{-1}) was used as a positive control. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as M $FeSO_4$ equivalents per gram of extracted compound.

Determination of 2, 2'-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity: The ABTS radical cation scavenging activity of ethyl acetate and acetone of flower extractes was determined by the method described by Re et al. [43]. 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 h. 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 min and then the absorbance was measured at 734 nm using digital spectrophotometer (Systronic, India), against a blank sample. Ascorbic acid was used as a positive control. Percentage inhibition was calculated.

RESULTS AND DISCUSSION

Plant products have been part of phytomedicine since time immemorial. These can be derived from any part of the plant i.e. any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances.

Extractive yield: Traditionally, water is used for extraction but since many secondary metabolites are not fully extracted in water, organic solvents are used. The extraction yield depends on type of solvents, time and temperature of extraction, as well as the chemical nature of the sample. Under the same time and temperature conditions, the solvent used and the chemical property of sample are the two most important factors. The extractive yield varied with different solvents in different flower extracts (Fig.1). In non polar solvent hexane, maximum extractive yield was in *A. scholaris* and minimum in *C. procera*. In semi polar solvent ethyl acetate, maximum extractive yield was in *Cassia auriculata* and minimum in *C. procera*. In polar solvent acetone, maximum extractive yield was in *C. auriculata* and minimum in *C. procera*. Efficiency of extraction is an important step involved in the discovery of bioactive components from plant material. Many solvents and different extracting techniques have been used for the extraction of bioactive compounds from plant material [44-46]. Different plants, different parts, different solvents give different yield [47] and similar results are reported here.

Quantitative phytochemical analysis

Total Phenol Content: The total phenol content varied with different solvents in different flower extracts. On the whole, the total phenol content was more in acetone extract than in ethyl acetate extract irrespective of the flower extract evaluated (Fig. 2). In ethyl acetate extract, maximum total phenol content was in *C. auriculata* and minimum in *C. procera* while in acetone extract, maximum total phenol content was in *C. auriculata* and minimum in *A. scholaris*. When all the 8 extracts are compared maximum phenol content was in *C. auriculata* (106.63) and minimum in *C. procera* (19.9). There are many reports in the literature that there is a direct correlation between phenol content and antioxidant activity [48, 49]. In fact, phenolic content is a good indicator of antioxidant capacity of a plant extract [50]. The flowers of *C. auriculata* with maximum phenol content can probably show good antioxidant activity while the flowers of *C. procera* may show less antioxidant activity.

Total Flavonoid Content: Flavonoids are a large group of naturally occurring plant phenolic compounds including flavonnones and chalcones. Many studies have suggested that flavonoids exhibit numerous biological activities, such as antiallergenic, antiviral, anti inflammatory, hepatoprotective, antioxidant, antithrombotic and anticarcinogenic activities [51]. Flavonoids like many other polyphenols are excellent free radical scavengers because they are highly reactive as hydrogen or electron donors [52].

The total flavonoid content varied with different solvents in different flower extracts. On the whole, the total flavonoid content was more in semi polar solvent ethyl acetate extract than in acetone extract irrespective of the flower extract evaluated except in *A. scholaris* (Fig. 3). In ethyl acetate extract, maximum total flavonoid content was in *C. roseas* and minimum in *C. auriculata* while in polar solvent acetone extract, maximum total flavonoid content was in *A. scholaris* and minimum in *C. auriculata*. When all the 8 extracts are compared maximum flavonoid content was in *C. roseas* and minimum in *C. auriculata*.

Antioxidant Activity: There are many methods used to evaluate the free radical scavenging activity of compounds [50]. The antioxidant activities of plant extracts vary with assay methods because of the complex nature of phytochemicals present in them, the solvent used for extraction, etc [53]. It is thus important that several analytical methods and different substrates are used for evaluating the effectiveness of antioxidants.

Ferric reducing antioxidant power (FRAP): The ferric reducing antioxidant power varied with different solvents in different flower extracts. On the whole, the FRAP was more in acetone extract than in ethyl acetate extract irrespective of the flower extract evaluated (Fig. 4). In semi polar solvent ethyl acetate extract, maximum FRAP was in *C. auriculata* and minimum in *A. scholaris* while in polar solvent acetone extract, maximum FRAP was in *C. auriculata* and minimum in *A. scholaris.* When all the 8 extracts are compared maximum FRAP activity was in *C. auriculata* (12.70) and minimum in *A. scholaris* (1.2). It is suggested that *C. auriculata* flower extract was able to reduce the complex ferric ion (Fe^{3+}) TPTZ to another complex ferrous ion (Fe^{2+}) TPTZ by releasing an electron, while flower extract of *A. scholaris* was unable to reduce ferric ion. There was a direct correlation between TPC and FRAP activity. Irrespective of the flower extract evaluated, acetone extracts had more TPC and more FRAP activity. However, when individual flower extract is compared both ethyl acetate extract had almost same amount of TPC and FRAP activity.It appears that both both phenolic and non phenolic compounds are responsible for antioxidant activity of this flower extract. Kaneria and Chanda[5] and Chandra et al. [54] also report that non phenolic compounds also may contribute for antioxidant capacity of plant extracts.

ABTS radical cation scavenging activity: The ABTS radical cation scavenging activity of different solvent extracts in different flower extracts is given in Figs. 5, 6. The activity was different in different flower extracts and also different in different solvents. The range of IC_{50} value was from 96 ug/ml to 392 ug/ml. Lowest IC_{5o} value was in ethyl acetate extract of *C. auriculata* (110 µg/ml) followed by ethyl acetate extract of *C. roseas* (150 µg/ml) (Fig. 6). The lowest IC_{50} value in acetone extract was in *C. auriculata* (96 µg/ml) followed by *C. roseas* (141 µg/ml). Overall, a moderate ABTS activity was shown by the flower extracts; but it is appreciable fact that even the extracts were crude they showed promising antioxidant activity and suggests that they can be further purified and also can be evaluated for other antioxidant activities. It can be concluded that non phenolic compound are also responsible for antioxidant activity as also suggested by Wong et al [55]. In the present work, *C. auriculata* had higher TPC in AC extract and FRAP activity and ABTS activity was more in AC extract (lowest IC_{50} value) while *C. procera* had lower TPC and lower FRAP and ABTS activity (higher IC_{50} value) indicating a positive correlation between phenolic content and antioxidant activity. The data also support the view that the flowers are promising source of natural antioxidants and could be seen as potential sources of useful drugs. Finally considering all aspects, it appears that *C. auriculata* was best among the four flowers screened and can be considered as a medicinal source for the treatment of many free radical related diseases.

Table.1. List of some Flowers, their family and their various activities.

No.	Botanical Name	Family	Activity	References
1	*Alcea hyrcana* Grossh	Malvaceae	Antioxidant	[29]
2	*Bidens tripartite* L.	Asteraceae	Antioxidant	[30]
3	*Caragana sinica (Buc'hoz)* Rehd	Fabaceae	Antioxidant	[31]
4	*Lantana camara* L.	Verbenaceae	Antioxidant	[32]
5	*Lippia alba*	Verbenaceae	Antioxidant	[33]
6	*Musa paradicicus*	Musaceae	Antioxidant	[34]
7	*Onopordon leptolepis* DC	Asteraceae	Antioxidant	[35]
8	*Salvia officinalis* L.	Lamiaceae	Antimicrobial	[36]
9	*Tagetes erecta*	Asteraceae	Antioxidant	[37]
10	*Thespesia populnea* (L.)	Malvaceae	Antibacterial	[38]

Please add figures here

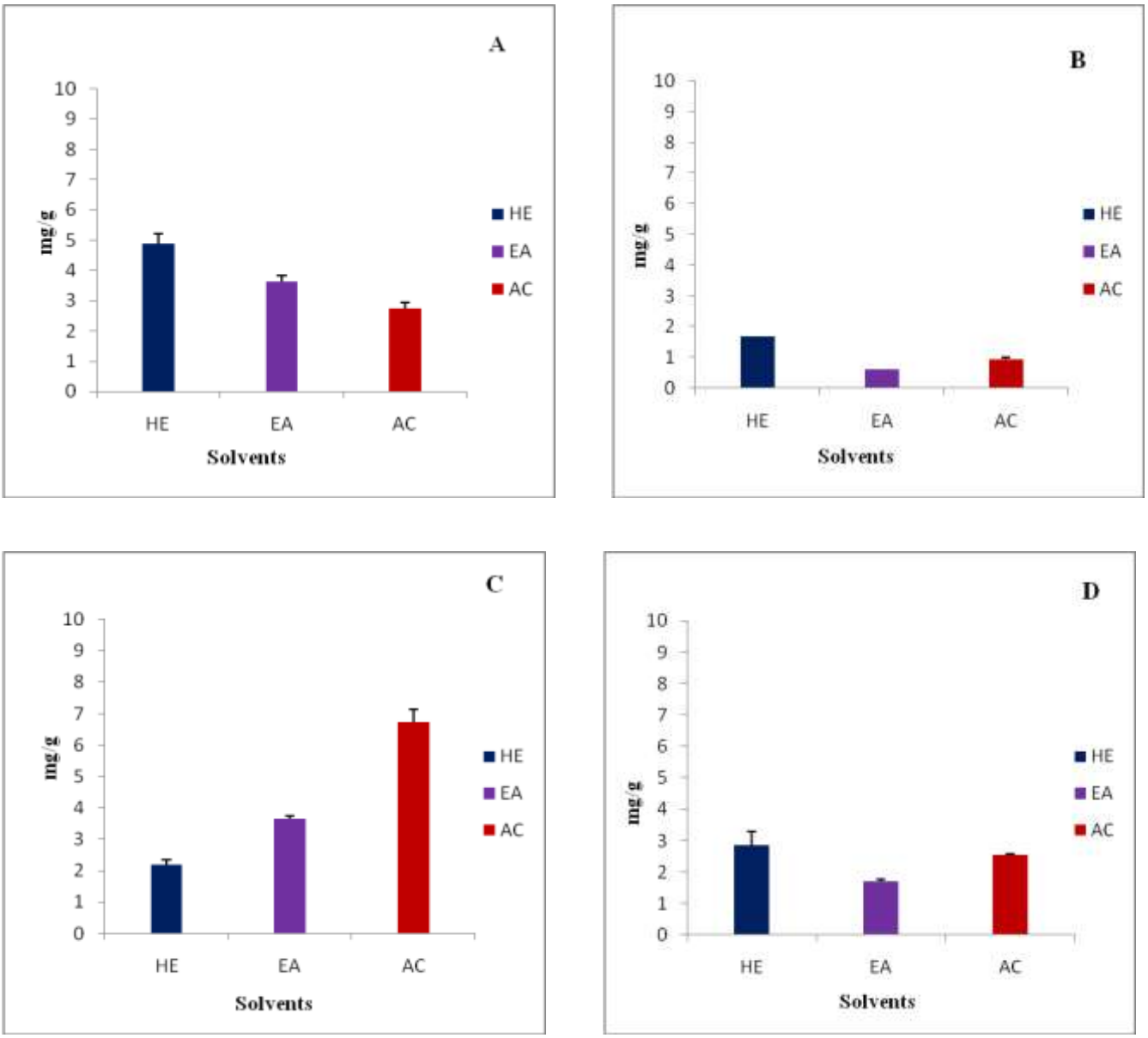

Fig.1 Extractive yield of different solvent extracts of flowers
A= *Alstonia scholaris*, B= *Calotropis procera*, C= *Cassia auriculata*, D= *Catharanthus roseas*

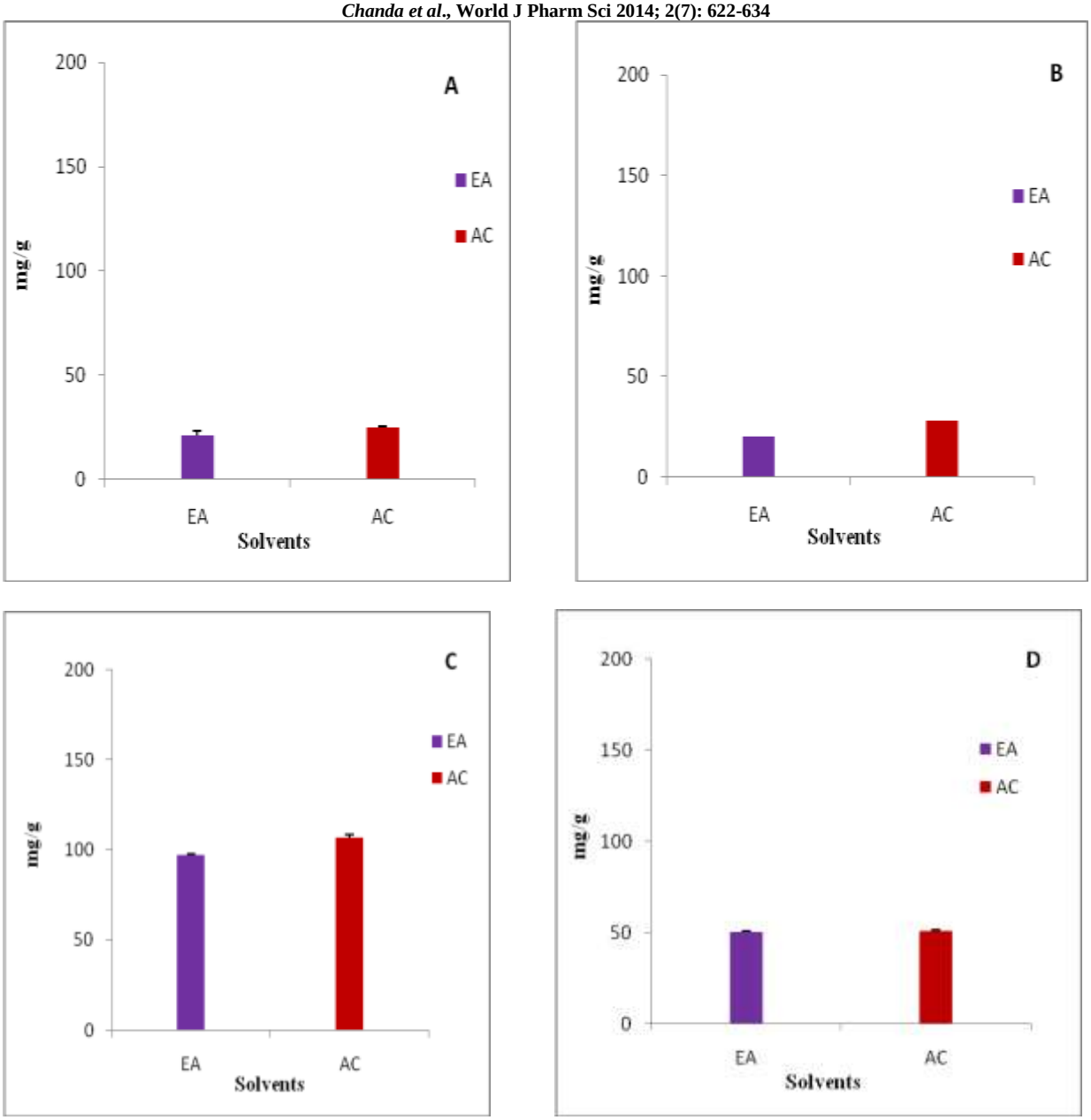

Fig.2 Total Phenol Content of different solvent extracts of flowers
A= *Alstonia scholaris*, B= *Calotropis procera*, C= *Cassia auriculata*, D= *Catharanthus roseas*

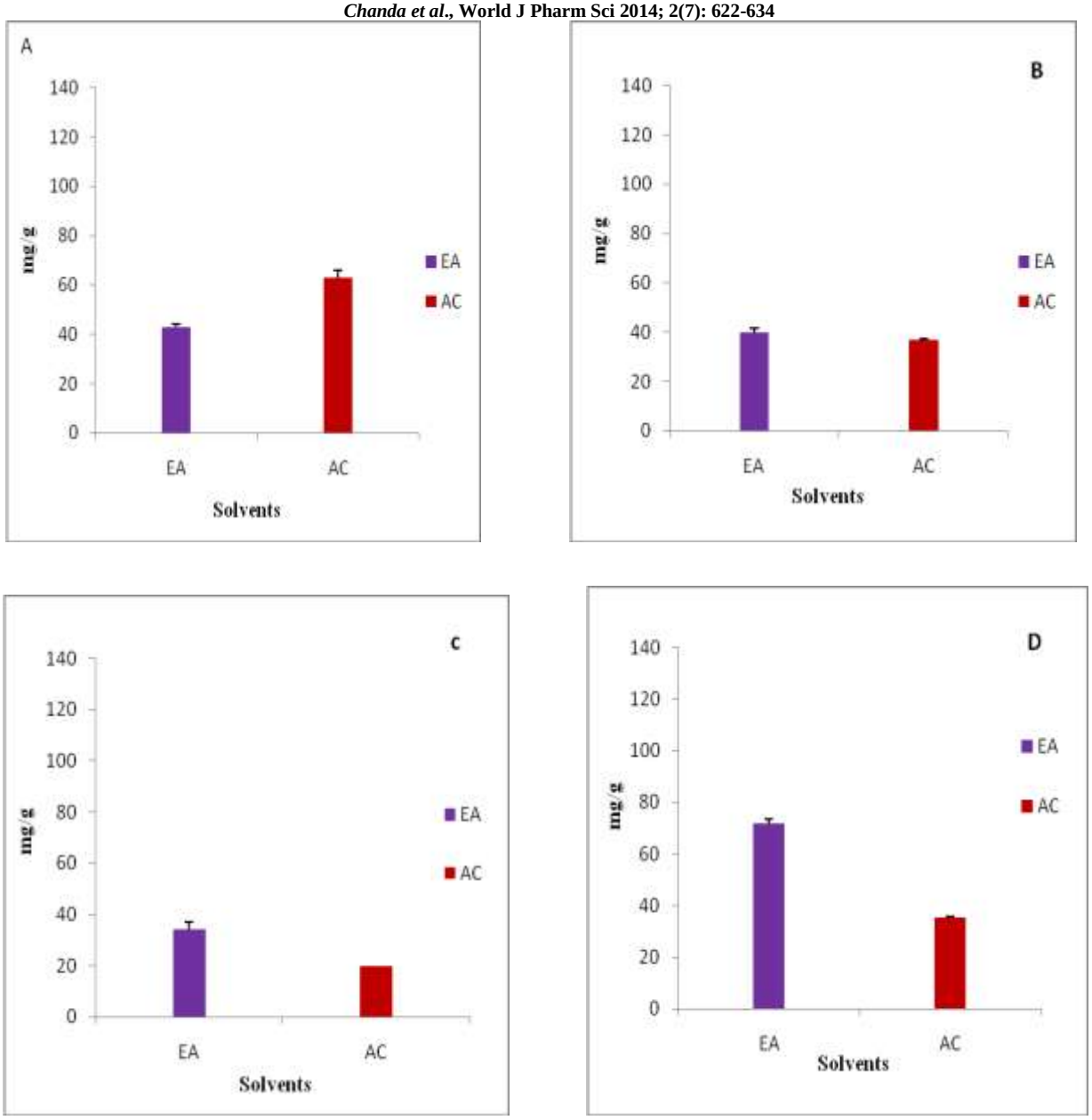

Fig.3 Total Flavanoid Content of different solvent extracts of flowers
A= *Alstonia scholaris,* B= *Calotropis procera,* C= *Cassia auriculata,* D= *Catharanthus roseas*

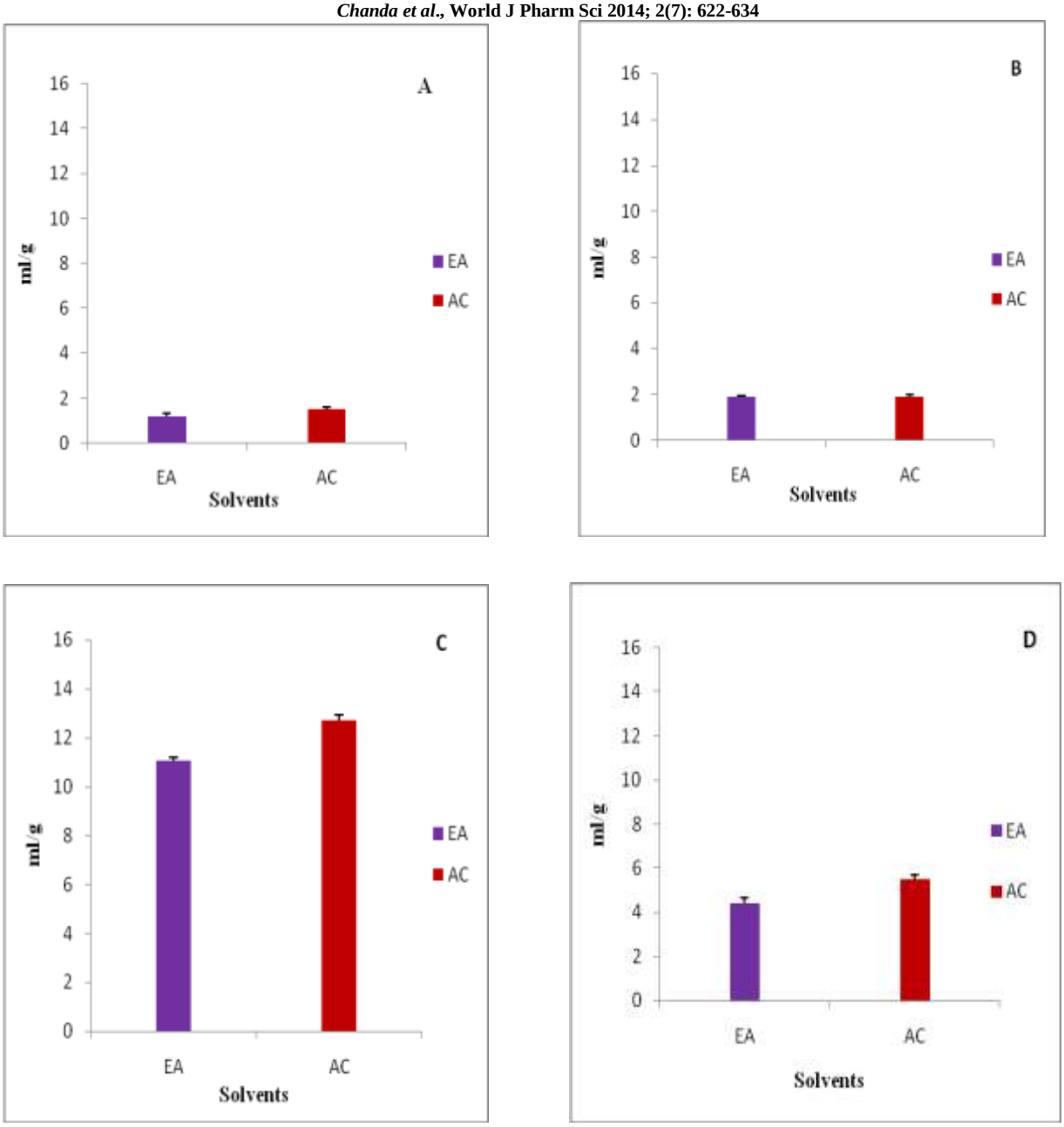

Fig. 4 Ferric reducing antioxidant power of different solvent extracts of flowers A= *Alstonia scholaris,* B= *Calotropis procera,* C= Cassia *auriculata,* D= *Catharanthus roseas*

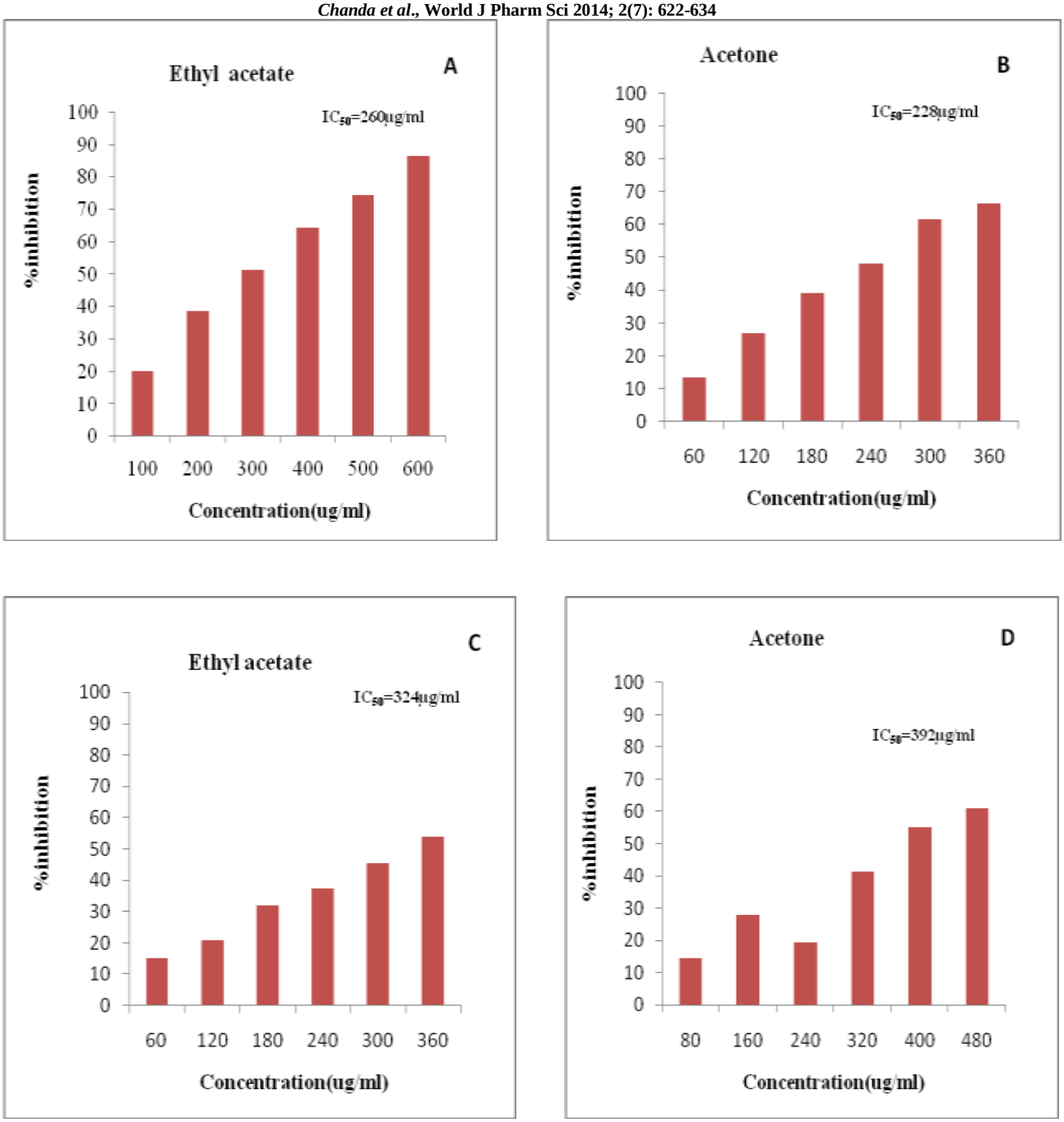

Fig.5 ABTS activity of different solvents extracts of A&B= *Alstonia scholaris* , C&D = *Calotropis procera*

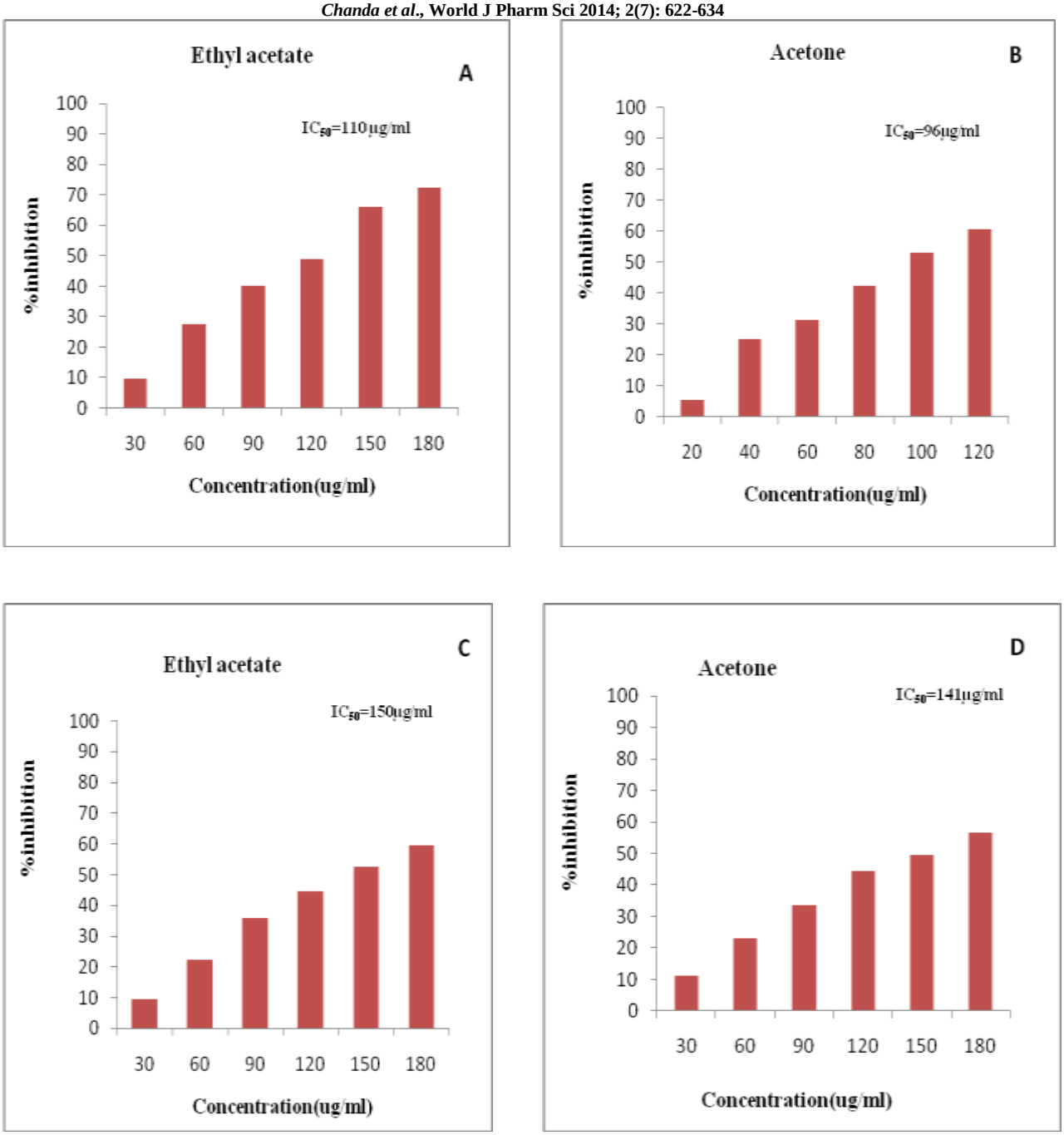

Fig.6 ABTS activity of different solvents extracts- A&B= *Cassia auriculata*, C&D = *Catharanthu roseas*

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