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Moteriya P
Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University - Rajkot, 360 005, Gujarat, India.

Ram J
Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University - Rajkot, 360 005, Gujarat, India.

Moradiya R
Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University - Rajkot, 360 005, Gujarat, India.

Chanda S
Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University - Rajkot, 360 005, Gujarat, India.

Correspondence:
Chanda S
Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University - Rajkot, 360 005, Gujarat, India.

In vitro free radical scavenging and antimicrobial activity of *Cyamopsis tetragonoloba* L

Moteriya P, Ram J, Moradiya R, Chanda S

Abstract
The aim of the present study was to evaluate the antioxidant and antimicrobial potential of different parts (leaf, stem and fruit) of a *Cyamopsis tetragonoloba* L. The extraction was done by cold percolation method using solvents like petroleum ether, ethyl acetate, acetone, methanol and water (aqueous). Total phenol and flavonoid content was measured by Folin- ciocalteu's reagent and aluminium chloride colorimetric method respectively. The antioxidant activity was evaluated using antioxidant assays like 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, superoxide radical scavenging assay (SO), 2, 2'-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS), Ferric Reducing Antioxidant Power (FRAP) and reducing capacity assessment. The antibacterial activity was done by agar well diffusion method against four Gram positive, four Gram negative bacteria and four fungi. The results suggest that phenolic and non phenolic compounds are responsible for antioxidant activity. The Gram positive bacteria were more susceptible than Gram negative bacteria and fungi. Different parts showed different levels of antioxidant activity but they can be explored as a natural source of antioxidants.

Keywords: *Cyamopsis tetragonoloba;* antioxidant activity; antimicrobial activity; total phenol content; total flavonoid content

Introduction
Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Medicinal plants are the best source to obtain a variety of drugs. Recently, there is a rise in the use of phyto pharmaceuticals as complementary or alternative medicine either to prevent or ameliorate many diseases mainly because the green medicine is considered as safe and free from many side effects which the synthetic drugs are known for.

Oxidative stress produces free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions but become deleterious when they are not eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. Free radicals harm healthy cells, create harmful molecules and contribute to the degenerative processes related to aging and diseases, e.g. cancer, cardiovascular disease and neurodegenerative disorders, such as Alzheimers disease (Shon *et al*., 2003) [20].

The antioxidants are capable of inhibiting the oxidation of biomolecules by removing free radical intermediates and inhibiting other oxidation reactions. Antioxidants could also interrupt peroxidation by donating hydrogen atom rapidly to a lipid radical, forming a new radical, more stable than the initial one oxidative stress occurs when there are low levels of antioxidants or inhibition of the antioxidant enzymes resulting in cell damage or cell death (Awah *et al*., 2012) [2].

Several commercially available synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ) are currently in use but their possible toxic properties for human health and environment is questionable (Harini *et al*., 2012) [8]. Therefore, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Natural antioxidant substances are presumed to be safe since they occur in plant foods and are more desirable than their synthetic counterparts. Natural antioxidants occur in all higher plants, and in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen and seeds).

In recent years, multiple drug resistance in human pathogenic microorganisms have developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment

of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources, like medicinal plants, which are the good sources of novel antimicrobial chemotherapeutic agents. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic and develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments.

Cyamopsis tetragonoloba L. is an herbaceous plant belonging to the family Fabaceae. *C. tetragonoloba* is rich in proteins, carbohydrates and the lack of toxins. It contains high amount of iron, minerals like sodium, potassium, calcium, magnesium, nickel, copper, cobalt and vitamins. It contains carotenoids like lutein, beta- carotene, violaxanthin and neoxanthin and flavonoids like quercetin, kaemferol, catechins, epicatechins and procyanidins (Laura *et al*., 2010) [11].

Guar beans have a large endosperm which contains galactomannan gum which forms a gel in water. Guar gum, a natural gum, is an edible thickening agent extracted from the guar bean, which is widely used in food and industrial applications. It is mainly used as a natural thickener, emulsifier, stabilizer, bonding agent, hydrocolloid, gelling agent, soil stabilizer, natural fibre, flocculants and fracturing agent. It is used as a laxative. It is also used for treating diarrhea, irritable bowel syndrome (IBS), obesity and diabetes, for reducing cholesterol and for preventing atherosclerosis.

In the present work, an attempt has been made to evaluate the antioxidant and antimicrobial properties of different parts (leaves, stem and fruit) of *C. tetragonoloba*.

Material and methods

Plant Collection

The plant *Cyamopsis tetragonoloba* L. was collected in August, 2014 from Morbi district, Gujarat, India. Different parts of the plant like leaf, stem and fruit were separated and then thoroughly washed with tap water and dried under shade. The dried parts were homogenized to fine powder and stored in air tight bottles which were later used for solvent extraction.

Extraction

The dry powder of leaf, stem and fruit of *C. tetragonoloba* was extracted individually by cold percolation method (Parekh and Chanda, 2007) [18] using different organic solvents like petroleum ether (PE), ethyl acetate (EA), acetone (AC), methanol (ME) and water (AQ).

Determination of total phenol (TPC) and flavonoid content (TFC)

The amount of total phenol and flavonoid content was determined by Folin-ciocalteu's reagent method (Mc Donald *et al.,* 2001) [14] and aluminium chloride colorimetric method (Chang *et al.,* 2002) respectively. The procedure followed is as described earlier (Kaneria *et al*., 2012) [10].

Antioxidants assays

The antioxidant activity of the different solvent extracts was evaluated by four different *in vitro* antioxidant assays like DPPH free radical scavenging activity, superoxide anion radical scavenging activity, ABTS cation free radical scavenging activity and Ferric reducing antioxidant power. The procedure followed is as described earlier (Kaneria *et al*., 2012) [10].

Antimicrobial activity

Antimicrobial activity was done by Agar well diffusion method (Perez *et al.,* 1990; Parekh and Chanda, 2007) [19, 18] against Gram positive bacteria, Gram negative bacteria and fungal strains. The procedure followed is as described earlier (Chanda *et al*., 2013) [4].

Microorganisms tested

The microorganisms were obtained from National Chemical Laboratory, Pune, India. The Gram-positive bacteria studied were *Bacillus cereus* (BC) ATCC11778, *Bacillus subtilis* (BS) ATCC6633 *Staphylococcus aureus* (SA) ATCC29737, and *Corynebacterium rubrum* (CR) ATCC14898. The Gram negative bacteria were *Escherichia coli* (EC) NCIM2931, *Pseudomonas aeruginosa* (PA) ATCC27S53, *Salmonella typhimurium* (ST) ATCC23564 and *Klebsiella pneumonia* (KP) NCIM2719. The fungal strains were *Candida albicans* (CA) ATCC209l, *Cryptococcus neoformans* (CN) NCIM3542, *Candida glabrata* (CG) NCIM3448 and *Candida epicola* (CN) NCIMB367.

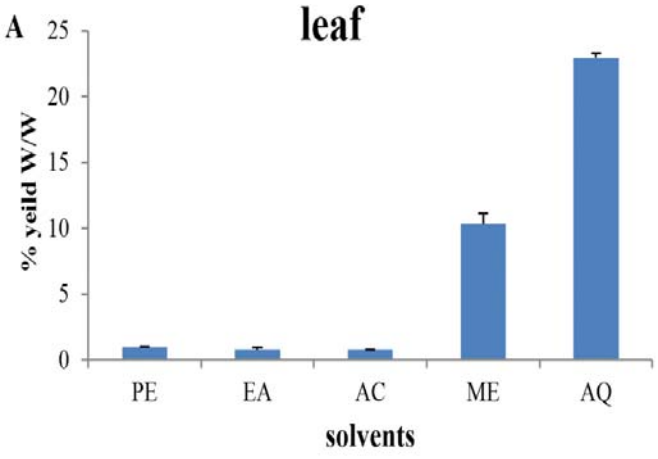
Statistical analysis

Each sample was analyzed individually in triplicate and the results are expressed as the mean value (n = 3) ± Standard Error of the Mean (S.E.M.).

Results and Discussion

Extraction yield

The extractive yield of different solvent extracts is given in Fig. 1. Among the three parts, maximum extractive yield was in leaf followed by fruit and stem respectively. In all the three parts, maximum extractive yield was in AQ extract followed by ME extract. In all the other three solvent extracts (PE, EA and AC) the extractive yield was very very less. The yield is different in different solvent extracts of different parts. This is very well reported because different parts or plants contain different phytoconstituents in different amounts and extraction yield depends on the polarity of the solvents and the nature of the constituents present in the part under investigation (Dash *et al*., 2005, Padalia *et al*., 2014) [7]. It is also apparent that higher or lower yield does not indicate it's antioxidant capacity or phenolic content.



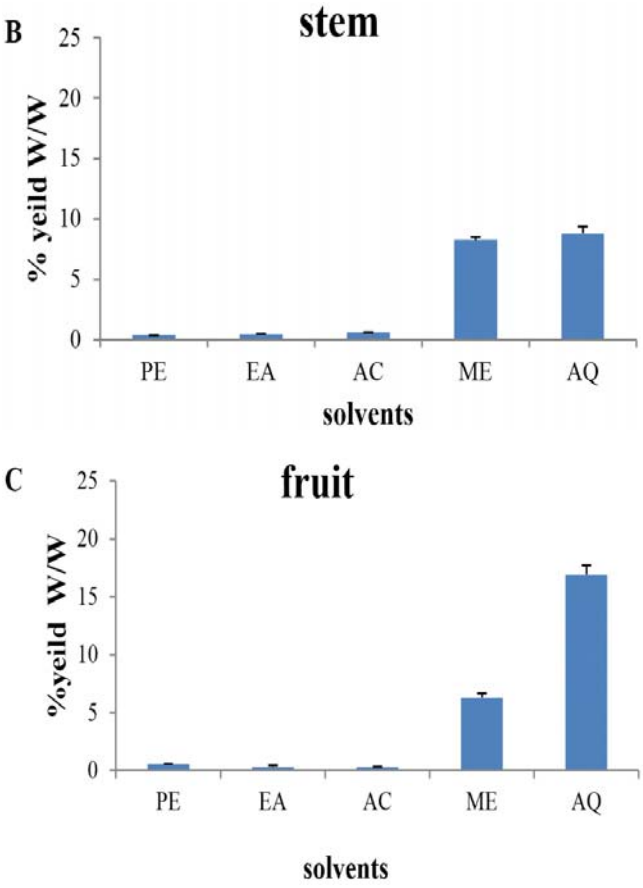


Fig 1: The extractive yield of different solvents extracts of *C. tetragonoloba* leaf (A), stem (B) and fruit (C)

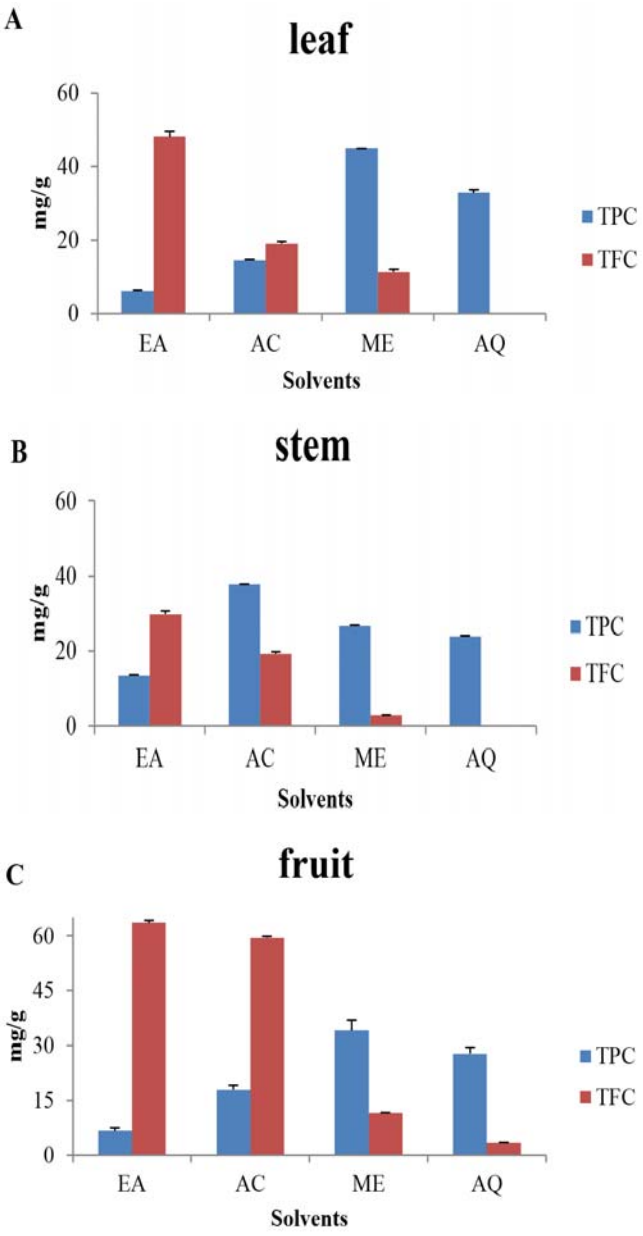


Fig 2: Total phenol content (TPC) and total flavonoid content (TFC) of different solvent extracts of *C. tetragonoloba* leaf (A), stem (B) and fruit (C)

Total Phenol Content (TPC) and total flavonoid content (TFC)

Plant polyphenols have several specific biological actions in preventing and or treating diseases. Flavonoids are ubiquitous polyphenolic metabolites in plants that have diverse beneficial biochemical and antioxidant effects (Dajas *et al.*, 2005) [6]. They have many health benefits mainly because of their antioxidant properties.

The TPC of different solvents extracts is given in Fig.2. Among the three parts, maximum TPC was in ME extract of leaf. In leaf, maximum TPC was in ME extract followed by AQ extract. In stem, maximum TPC was in AC extract followed by ME and AQ extracts. In fruit, maximum extractive yield was in ME extract followed by AQ extract. Thus it appears that methanol is a better solvent than other solvents for extracting phenolic compounds from different parts of the plant due to its polarity and good solubility for phenolic compounds. Similar results were reported by Kallel *et al*., (2014) [9], Moteriya *et al.*, (2014) [15] and Ammar *et al*., (2015) [1].

The TFC of different solvents extracts is given in Fig.2. In all the three parts, maximum TFC was in EA extract; maximum was in fruit. Among polar solvent AC and ME extracts, TFC was more in AC extract than in ME extract in all the three parts; maximum content was in AC extract of fruit. TFC was absent in leaf and stem AQ extracts; except AQ extract of fruit in which slight amount of TFC was present. In all the three parts EA extracts had maximum TFC. Moteriya *et al*., (2015) [16, 17] also reported maximum TFC in EA extracts of some flowers.

DPPH radical scavenging activity

DPPH radical is one of the routinely used antioxidant assay. This method is widely accepted because it is independent of sample polarity extracts and is effective while screening many samples (Magalhaes *et al*., 2008) [12]. The IC_{50} values of DPPH radical cation scavenging activity is given in Table 1. Amongst all the three parts, leaf ME extract had lowest IC_{50} value (350 µg/ml) followed by fruit AQ extract (600 µg/ml). In stem EA and AC extracts had almost same IC_{50} values followed by EA leaf extract (Table 1). Amongst the three parts, leaf ME extract showed good DPPH radical scavenging activity. It showed a direct correlation with TPC as also reported by Babbar *et al*., (2014) [3].

Super oxide anion scavenging assay

Super oxide anion radical scavenging activity of all the solvent extracts of the three parts is given Table 1. Out of 12 extracts

only 4 extracts i.e. AQ extracts of all the three parts and AC extract of leaf showed super oxide anion radical scavenging activity. The best activity was shown by AC extract of leaf. There was no correlation between TPC and this antioxidant activity in any part or any solvent extract. This is in contrast to DPPH activity indicating that it is not necessary that all the antioxidant assays show similar correlation with TPC because the mechanism of action is different in different assays.

The ABTS radical cation scavenging activity

ABTS radical cation scavenging activity is most popular spectrophotometric methods for determination of the antioxidant capacity of medicinal plant extracts. The ABTS radical cation scavenging activity is given in Table 1.

Among three parts, EA and AC extracts showed good ABTS activity. The AC extract of stem (59 µg/ml) showed lowest IC_{50} value and AC extract had maximum TPC i.e. it showed a direct correlation with TPC. The leaf and stem AQ extracts showed poor ABTS activity similar to that of DPPH activity. In both the antioxidant assays, only fruit AQ extract showed some scavenging activity. When DPPH and ABTS antioxidant activity of all the solvent extracts of the three parts is compared, a direct correlation between DPPH and ABTS was envisaged.

Ferric reducing antioxidant power (FRAP)

The FRAP assay measure the change in absorbance at 593 nm due to the formation of a blue colored complex of ferrous ion (fe^{2+}) and TPTZ. Prior to this a colorless ferric ion (fe^{3+}) gets oxidized to ferrous ion (fe^{2+}) by the action of electron donating antioxidants.

The FRAP of different solvent extracts is given in Table 1. Among the three parts, maximum FRAP activity was in leaf. In leaf, ME extract had maximum FRAP activity followed by AQ extracts. In stem, maximum FRAP activity was in AC extract followed by ME extract. In fruit, maximum FRAP activity was in AQ extract followed by ME extract. There was a direct correlation between TPC and FRAP activity in all the solvent extracts in all the three parts (leaf, stem and root) except fruit. Mak *et al*., (2013) [13] and Yang *et al*., (2015) [21] also reported a positive correlation between antioxidant activity and total phenol content.

Table 1: Antioxidant activity of different parts [leaf (A), stem (B) and fruit (C)] of *C. tetragonoloba*

No.	Solvents	DPPH IC_{50} value (µg ml^{-1})			SO IC_{50} value (µg ml^{-1})			ABTS IC_{50} value (µg ml^{-1})			FRAP activity (M/g)		
		A	B	C	A	B	C	A	B	C	A	B	C
1	EA	840	615	>1000	>1000	>1000	>1000	318	75	552	3.4	2.3	1.7
2	AC	>1000	637	>1000	320	>1000	>1000	476	59	472	3.8	3.9	1.8
3	ME	350	>1000	>1000	>1000	>1000	>1000	690	380	>1000	4.9	3.1	2.4
4	AQ	>1000	>1000	600	424	472	336	>1000	>1000	356	4.4	2.9	3.1

Different parts showed different levels in different radical scavenging activity. Irrespective of the part and the activity, the polar solvent extracts showed good scavenging capacity. The difference in antioxidant activity of different parts may be because of the different levels of phytoconstituents present in them. However, it can be stated all the parts possess some antioxidant properties which can be exploited for use as natural source of antioxidants.

Antimicrobial activity

The antimicrobial activity of different solvent extracts is given in Table 2-4. Different solvent extracts of different parts showed different levels of inhibition. All solvent extracts of all four parts showed very poor antimicrobial activity against tested microbes. In Gram positive bacteria, *C. rubrum* and *S. aureus* were most susceptible bacteria. It was inhibited by all the solvent extracts of all the three parts. All solvent extracts showed very poor antibacterial activity against *B. cereus* and *B. subtilis*. In Gram negative bacteria, *E. coli* was inhibited by some solvent extracts of leaf. *P. aeruginosa*, *K. pnemoniae* and *S. typhimurium* were highly resistant to all the other solvent extracts. In fungi, some of the extracts of stem and fruit showed antifungal activity against *C. albicans* and *C. neoformans. C. epicola* and *C. glabrata* were highly resistant to all the solvent extracts of all the three parts.

Table 2: Antimicrobial activity of *C. tetragonoloba* leaf

No.	Solvents	Gram positive bacteria				Gram negative bacteria				Fungi			
		BC	BS	CR	SA	EC	PA	KP	ST	CA	CG	CN	CE
1	PE	8.5	8.5	10	10	11.5	_	_	_	_	_	_	_
2	EA	9	8.5	10	12.5	10.5	_	_	_	_	_	_	_
3	AC	10	8.5	10	10.5	_	_	_	_	_	_	11	_
4	ME	8.5	8.5	11	10.5	_	10	_	_	_	_	_	_
5	AQ	_	_	_	_	_	_	_	_	_	_	_	_

Table 3: Antimicrobial activity of *C. tetragonoloba* stem

No.	Solvents	Gram positive bacteria				Gram negative bacteria				Fungi			
		BC	BS	CR	SA	EC	PA	KP	ST	CA	CG	CN	CE
1	PE	8.5	8.5	12	9.5	10.5	_	_	_	_	_	_	_
2	EA	10.5	8.5	11.5	11	_	_	_	_	10	_	13	_
3	AC	10.5	8.5	11.5	10	_	_	_	_	11	_	12	_
4	ME	8.5	_	_	10.5	_	_	_	_	9	_	10	_
5	AQ	_	_	11.5	10.5	_	_	_	_	_	_	_	_

Table 4: Antimicrobial activity of *C. tetragonoloba* fruit

No.	Solvents	Gram positive bacteria				Gram negative bacteria				Fungi			
		BC	BS	CR	SA	EC	PA	KP	ST	CA	CG	CN	CE
1	PE	8.5	8.5	10	_	_	_	_	_	_	_	_	_
2	EA	7.5	8.5	11.5	10	_	_	_	_	10	_	9	_
3	AC	9	9	10.5	10	_	_	_	_	12	_	_	_
4	ME	8.5	_	11	10	_	_	_	_	9	_	_	_
5	AQ	8.5	9	11.5	_	_	_	_	_	_	_	10	_

Conclusion

The results suggest that polarity of solvents used for extraction greatly influences the extraction of phytoconstituents and hence the antioxidant capacity. All solvent extracts of different parts of same plant may show a positive correlation between total phenolic content and antioxidant activity or some parts may show and some may not since phenolic and non phenolic compounds may be responsible for antioxidant activity. It may be part specific. Plant extract which show good antioxidant activity need not show good antibacterial activity. Different parts of *Cyamopsis tetragonoloba* showed different antioxidant activities hence this plant parts can further be exploited as a natural source of antioxidants and can be useful in treating oxidative stress related diseases.

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References

1. Ammar I, Ennouri M, Attia H. phenolic content and antioxidant activity of cactus (*Opuntia ficus*-indica L.) flowers are modified according to the extraction method. Indus Crops Prod 2015; 64:97-104.
2. Awah FM, Offor NN, Ndunaka AC, Okofor FU, Enyabine CO. Free radical scavenging activities and phenolic contents of the species *Thymus vulgaris* (Thyme), *Helichrysum italicum* (Curry leaf) and *Laurus nobilis* (Bay leaf) extracts. J Pharm Res 2012; 5:2994-2998.
3. Babbar N, Oberoi HS, Sandhu SK, Bhargav VK. Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. J Food Sci Technol 2014; 51(10):2568-2575.
4. Chanda S, Rakhloiya K, dholakia K, Baravalia Y. Antimicrobial, antioxidant and synergistic properties of two nutraceutical plants: *Terminali catappa* L. and *Colocasia esculenta* L. Turk J Biol 2013; 37:81-91.
5. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propoils by two complementary colorimetric methods. J Food Drug Anal 2002; 10:178-182.
6. Dajas F, Arredondo F, Echeverry C, Ferreira M, Morquio A, Rivera F. Flavonoids and the brain; evidences and putative mechanism for a protective capacity. *Curr Neuropharmacol* 2005; 3:193-205.
7. Dash S, Nath LK, Bhise S, Bhuyan S. Antioxidant and antimicrobial activities of *Heracleum nepalense* D Don root. Trop J Pharmaceut Res 2005; 4(1):341-347.
8. Harini R, Sindhu S, Sagadevan E, Arumugam P. Characterization of *in vitro* antioxidant potential of *Azadirachta indica* and *Abutilon indicum* by different assay methods. J Pharm Res 2012; 5:3227-3231.
9. Kallel F, Driss D, Chaari F, Belghith L, Bouaziz F, Ghorbel R *et al.* Garlic (*Allium sativum* L.) husk waste as a potential source of phenolic compounds: influence of extracting solvents on its antimicrobial and antioxidant properties. Inuds Crops Prod 2014; 62:34-41.
10. Kaneria M, Kanani B, Chanda S. Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Indian medicinal plant. Asian Pac J Trop Bio Med 2012; 2(3):195-202.
11. Laura A, Rosa DL, Parrilla EA, González GA. *Wiley Blackwell Publication* USA 2010; 1:53-88.
12. Magalhaes LM, Segundo MA, Reis S, Lima JLFC. Methodological aspects about *in vitro* evaluation of antioxidant properties. Anal Chim Acta 2008; 613:1-19.
13. Mak WY, Chuah LO, Ahmad R, Bhat R. Antioxidant and antimicrobial activities of hibiscus (*Hibiscus rosa-sinensis* L.) and Cassia (*Senna bicapsularis* L.) flower extracts. J King Saud Uni Sci 2013; 25:275-282.
14. Mc Donald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chem* 2001; 73:73-84.
15. Moteriya P, Ram J, Rathod T, Chanda S. *In vitro* antioxidant and antibacterial potential of leaf and stem of *Gloriosa superba* L. Am J Phytomed Clin Therapeut 2014; 2(6):703-718.
16. Moteriya P, Satasiya R, Chanda S. Screening of phytochemical constituents in some ornamental flowers of Saurashtra region. *J* Phcog Phytochem 2015; 3:112-120.
17. Padalia H, Moteriya P, Satasiya R, Chanda S. *In vitro* free radical scavenging activity *Nerium indicm, Peltophoram pterocarpum* and *Rosa* spps. flower extracts. Asian J Pharmaceut Clin Res 2015; 8(1):91-97.
18. Parekh J, Chanda S. *In vitro* antibacterial activity of the crude methanol extract of *Woodfordiafructicosa*kurz. flower (Lythraceae). *Braz J Microbiol* 2007; 38:204-207.
19. Perez C, Paul M, Bazerque P. An antibiotic assay bye the agar well diffusion method. Acta Biol Med Expt 1990; 15:113-115.
20. Shon MY, Kim TH, Sung NJ. Antioxidants and free radical scavenging activity of *Phellinus baumii* (Phellinus of Hymenochaetaceae) extracts. Food Chem 2003; 82:593-597.
21. Yang M, Shen Q, Li LQ, Huang YQ, Cheung HY. Phytochemical profiles, antioxidant activities of functional herb *Abrus cantoniensis* and Abrus mollis. Food Chem 2015; 177:304-312.