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Quantitative phytochemical analysis of Ten Medicinal Plants of Patna district

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Abstract
In the present study,10 medicinally important plant species were screened for their phytochemicals quantitatively by using ethanolic extracts from their selected plants parts. All the medicinal plants selected for the study contained significant amount of phytochemicals like alkaloids, flavonoids, phenols, saponins and Tannins. The amount of flavonoids was maximum in comparison to other phytochemicals in all the ten medicinal plants. In *Azadirachta indica* and *Syzygium aromaticum* the amount of flavonoids was highest in their ethanolic extract (47.75 QE and 47.35 QE and respectively), followed by *Anisomeles indica* and *Bauhinia variegata* (45.35 QE and 44.47 QE respectively), and *Oxalis corniculata* and *Ocimum sanctum* contains the same amount of flavonoid (42.75 QE), *Solanum nigrum*, *Coriandrum sativum, Terminalia bellirica, and Citrus sinensis* (41.35 QE to 40.55 QE).

Keywords: Phytochemical, Amount, flavonoid and Medicinal Plant

Introduction
Plants have been appreciated for their antimicrobial or medicinal properties for centuries. Nature has long been a great source of antimicrobial compounds and play important role in natural defense and thus this is logical to search natural alternatives to chemical one. Compounds that are present in plants have antimicrobial activity and called as green chemicals are either proteins, hydrolytic enzymes or secondary metabolites from the terpenoid or phenolic biosynthesis origin (Vigers et al., 1991) [1].

The use of numerous plant extracts, various spices and their constituents provide an alternative way to prevent fungal growth and mycotoxins formation (Vagi et al., 2005) [2]. In this era of antimicrobial drug resistance, some plants have antimicrobial potential and is considered as alternatives to conventional antimicrobial agents (Nwaopara et al., 2009) [3]. Plants can synthesize aromatic secondary metabolites, like phenols, quinones, flavones, phenolic acids, flavonoids, flavonols, coumarins and tannins (Cowans, 1999). [4]

The plant compounds which are secondary metabolites, mainly of terpenoid or phenolic biosynthetic origin, hydrolytic enzymes (Glucanases, chitinases) and proteins acting specifically on membranes of invading microorganisms with antimicrobial activity (Bowles, 1990; Vigers et al., 1991). [5, 1] No sharp chemical division can be made in general between constitutive and induced antimicrobials.

The extracts of different parts of medicinal plants parts like root, stem, flower and fruit were widely used for treatments of some human diseases (Khan et al., 2013) [6]. Medicinal plants contain several phytochemicals namely alkaloids, flavonoids, tannins, and terpenoids which possess antimicrobial and antioxidant properties (Talib and Mahasneh, 2010) [7].

Phytoalexins, organic acids, essential oils and phenolic compounds are the main groups of plant derived antimicrobial compounds considered for food preservation purposes (Gould, 1996b; Smid and Gorris, 1999) [8, 9]. Phenolic compounds are a wide group of antimicrobials which also include some plant extracts, essential oils (Nakatani, 1994) [10] and phytoalexins (Smid and Gorris, 1999) [9].

Materials and Methods
- Ten different medicinal plants namely: *Syzygium aromaticum* (Clove), *Oxalis corniculata* (Wood sorel), *Solanum nigrum* (Black nightshade), *Azadirachta indica* (Neem), *Bauhinia variegata* (Kachnar), *Coriandrum sativum* (Dhania), *Anesomeles indica* (Indian catmint), *Terminalia bellirica* (Bahera), *Ocimum sanctum* (Tulsi) and *Citrus cinensis* (Orange) were collected in their natural habitat from local areas of Patna. The plant was authenticated by taxonomists from Botany Department, P.U., Patna.

- The samples were cut into small pieces and then surface sterilized with 5% Sodium hypochlorite solution. The plant parts were dried in shade for 48 hours approximately at ambient temperature under laboratory condition and then crushed to fine powder in electric grinder. These were sealed in polyethylene bags and stored away from light and moisture until used for extraction (Yaye et al., 2012) [11].

Preparation of ethanolic extract of plant sample:
The ethanolic extract was prepared by soaking 10 gm of powdered plant parts in 100 ml of ethanol for the same 12 hrs. The extracts were then filtered using filter paper. The extracts were then concentrated to 50 ml of the extracts sample and stored in airtight container.

Determination of Alkaloids
Alkaloid content was measured by method suggested by Harborne (Harborne, 1973) [12]. A suspension was prepared by dispersing 5 gm of the plant parts in 10% acetic acid solution in ethanol and kept at 28 ^{0}C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous NH_4OH were added. Finally, the precipitate was washed with 1% ammonia solution and dried at 80^{0}C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample.

Determination of Tannins
The quantitative estimation of tannins was performed by the method of Swain (Swain, 1979) [13] with minor modifications. The finely powdered plant parts were kept in a beaker containing 20 ml of 50% methanol covered with parafilm and then heated at 80 ^{0}C in a water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and rinsed with 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2CO_3 for the development of a bluish-green colour and was allowed to stand for 20 mins. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

Determination of Saponins
Saponin analysis was performed according to the method described by Brunner (Brunner, 1984) [14]. 100 ml Isobutyl alcohol was added to 1 gm of the finely powdered sample and stirred for 5 hrs. 20 ml of 40% saturated solution of Magnesium carbonate was added to the mixture and filtered. 2 ml of 5% $FeCl_3$ solution and 50ml volume of distilled water was added to 1ml of colourless solution and kept for 30 mins for colour (blood red) development The absorbance of the samples as along with the standard were read at 380 nm and calculated in mg/g. Standard saponin solution was prepared in the reference range of 0-10 ppm.

Total Phenolics determination
The amount of total phenolics in aqueous and ethanol extracts was determined by the Folin–Ciocalteu method (Folin & Ciocalteu, 1927) [15]. Gallic acid was used as a standard by using different concentrations of (20-200µg) from which the total phenol content in the extract was expressed in terms of gallic acid equivalent (mg GAE/gm) extract. Different aliquots of 0.1 to 1.0 ml of plant extract were also prepared in methanol and 0.5 ml of each sample were introduced into test tubes and mixed with 2.5 ml of a 10-fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30 mins at room temperature. Phenols react with the phosphomolybdic acid in Folin- Ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue). The absorbance of the resulting solutions was measured at 760 nm against reagent blank. A standard calibration curve was prepared by plotting absorbance against concentration and it was found to be linear over this concentration range. The concentration of total phenol in the test sample was determined from the calibration graph. The assay was carried out in triplicate and the mean values with ± SD are presented.

Total Flavonoids determination
The aluminium chloride colorimetric method was used for flavonoids determination (Chang *et al.,* 2002) [16]. The aqueous and ethanol plant extracts were separately mixed with 1.5 ml of ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 418 nm. The percentage of total flavonoids were calculated from the calibration curve of Quircetin (200-1000µg) plotted by using the same procedure and total flavonoids was expressed Quircetin equivalents (QE) in mg per gm sample.

Results and Discussion
The phytochemicals of ten selected medicinal plants viz. *Syzygium aromaticum* (Clove), *Oxalis corniculata* (Wood sorel), *Solanum nigrum* (Black nightshade), *Azadirachta indica* (Neem), *Bauhinia variegata* (Kachnar), *Coriandrum sativum* (Dhania), *Anesomeles indica* (Indian catmint), *Terminalia bellirica* (Bahera), *Ocimum sanctum* (Tulsi) and *Citrus sinensis* (Orange) were analysed quantitatively in their ethanolic extracts from their respective parts and the results obtained have been presented in Table 1.
In the ethanolic extract of leaves of *Syzygium aromaticum* and *Azadirachta indica* the amount of alkaloid was maximum (21.45 mg/gm) followed by Solanum nigrum (20.75 mg/gm), *Citrus sinensis* (20.65 mg/gm), *Anesomeles indica* (19.65 mg/gm) and *Coriandrum sativum* (19.37 mg/gm). In T*erminalia bellirica, Bauhinia variegata*, *Oxalis corniculata* and *Ocimum sanctum* the amount of alkaloid was relatively less in the range of 18.85 mg/gm, 18.75mg/gm, 18.65mg/gm, 18.45 mg/gm, respectively. In *Azadirachta indica* and *Syzygium aromaticum* the amount of flavonoids was high in their ethanol extract (47.75 QE and 47.35 QE and respectively), followed by *Anisomeles indica* and *Bauhinia variegata* (45.35 QE and 44.47 QE respectively), and *Oxalis corniculata* and *Ocimum sanctum* contains the same amount of flavonoid (42.75 QE), *Solanum nigrum*, *Coriandrum sativum, Terminalia bellirica, and Citrus sinensis* (41.35 QE to 40.55 QE). The total phenolic compounds were maximum in *Azadirachta indica* and *Syzygium aromaticum* (24.85 GAE and 23.75 GAE respectively), followed by *Anesomeles indica, Citrus sinensis* and *Solanum nigrum*, (22.36 GAE, 22.35 GAE and 22.25 GAE respectively). *Terminalia bellirica, Oxalis corniculata, Bauhinia variegata, Coriandrum sativum* and *Ocimum sanctum* contained relatively lesser amount of phenolic compounds in the range of 21.67 GAE to 21.35 GAE. The amount of saponin was maximum in *Oxalis corniculata* (18.35 GAE) followed by *Syzygium aromaticum, Solanum nigrum, Terminalia bellirica* (17.85 mg/gm, 16.45

mg/gm and 16.37 mg/gm respectively), followed by *Ocimum sanctum, Anisomeles indica, and Citrus sinensis, Bauhinia variegata* (15.67 mg/gm,15.65 mg/gm, and 15.65 mg/gm,15.35 mg/gm, respectively), and *Azadirachta indica* and *Coriandrum sativum* (14.35 mg.gm and 14.75 mg/gm respectively). The amount of tannins was maximum in *Azadirachta indica* and *Bauhinia variegata* (16.75 mg/gm and 16.35 mg/gm respectively) The amount of Tannin was equal in *Syzygium aromaticum*, *Citrus sinensis* (15.45 mg/gm) followed by *Terminalia bellirica* (15.25 mg/gm), *Anisomeles indica* (14.75mg/gm) *Ocimum sanctum*(14.55mg/gm), *Coriandrum sativum*(14.45mg/gm), *Solanum nigrum* (14.35 mg/gm). *Oxalis corniculata* contained lesser amount of tannin (13.45 mg/gm) (Table 1; Fig A).

Table 1: Quantitative estimation of phytochemicals in ten medicinal plants in their ethanol extracts (amount in mg/gm ±SE; Total Phenol in GAE in mg/100gm; Flavonoids in QE in mg/100gm).

Sl. No.	Medicinal plants	Alkaloids	Flavonoids	Total Phenol	Saponins	Tannins
1	*Syzygium aromaticum* (Clove)	21.45 ±0.11	47.35 ±0.45	23.75 ±0.32	17.85 ±0.22	15.45 ±0.21
2	*Oxalis corniculata* (Creeping wood sorel)	18.65 ±0.50	42.75 ±0.41	21.65 ±0.33	18.35 ±0.27	13.45 ±0.36
3	*Solanum nigrum* (Black Nightshade)	20.75 ±0.41	41.35 ±0.51	22.25 ±0.44	16.45 ±0.23	14.35 ±0.26
4	*Azadirachta indica* Neem	21.45 ±0.47	47.75 ±0.37	24.85 ±0.34	14.35 ±0.21	16.75 ±0.27
5	(*Bauhinia variegata*) Kachnar	18.75 ±0.63	44.47 ±0.31	21.65 ±0.32	15.35 ±0.21	16.35 ±0.21
6	*Coriandrum sativum* (Coriander)	19.37 ±0.45	40.55 ±0.51	21.35 ±0.21	14.75 ±0.16	14.45 ±0.18
7	*Anesomeles indica* (Indian catmint)	19.65 ±0.32	45.35 ±0.31	22.36 ±0.21	15.65 ±0.24	14.75 ±0.41
8	*Terminalia bellirica* (Bahera)	18.85 ±0.37	41.25 ±0.33	21.67 ±0.18	16.37 ±0.15	15.25 ±0.41
9	*Ocimum sanctum* (Tulsi)	18.45 ±0.32	42.75 ±0.37	21.65 ±0.41	15.67 ±0.21	14.55 ±0.16
10	*Citrus sinensis* (Orange)	20.65 ±0.25	41.25 ±0.61	22.35 ±0.37	15.65 ±0.19	15.45 ±0.31

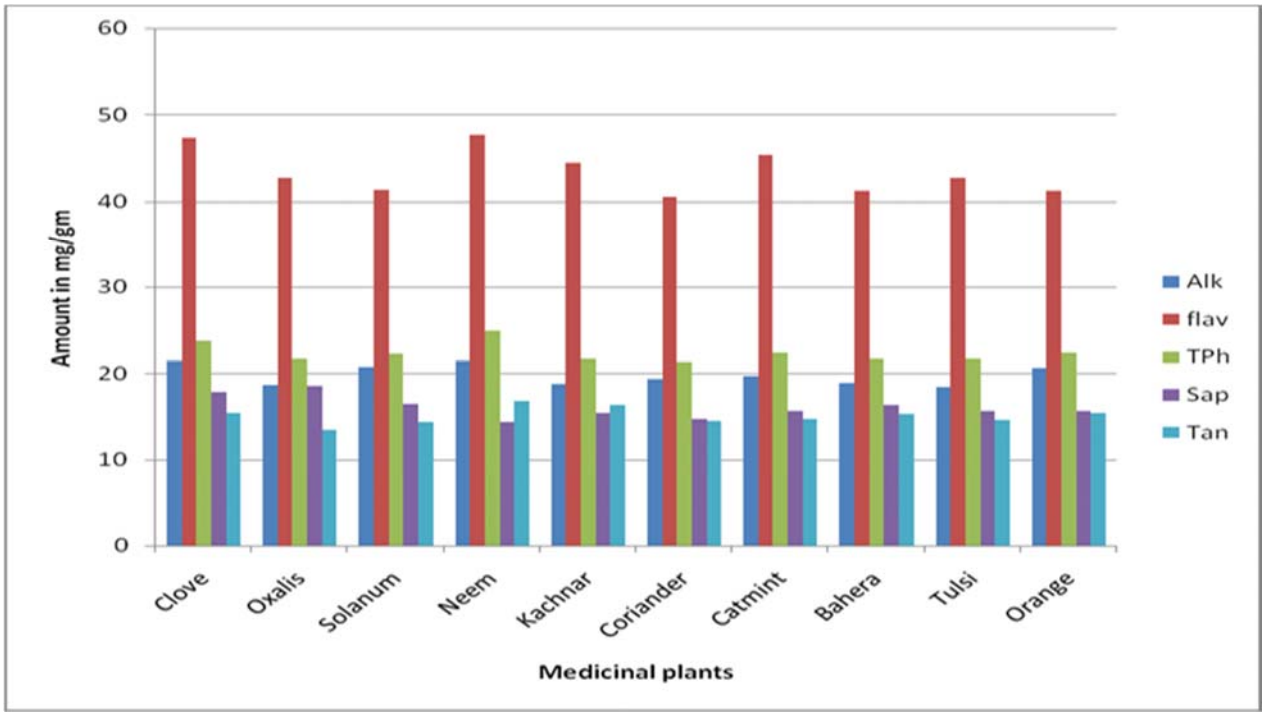

Fig A: Quantitative value of phytochemicals in ten medicinal plants in ethanolic extract. Total phenol in GAE in mg/100gm: Flavonoids in QE in mg/100g. Alk: Alkaloid; Flv: flavonoids; Tph: Total phenol; Sap: Saponins; Tan: Tannins

Phytochemicals of medicinal plants and their medicinal values have been studied by many workers. Emmanuel *et al.*, (2015) [17] have studied the phytochemicals of *Syzygium aromaticum* flower and found more or less similar results. A more or less similar phytochemical constituent from clove has also been investigated by Rashi *et al.*, (2011) [18] and Umesh Kumar *et al*., (2010) [19]. Nirmala Paul *et al.*, (2013), Nimish *et al*., (2011), Arun thangavel *et al.*, (2015), Sasi Kumar *et al*., (2014) [20-23] have investigated the similar phytochemicals in *Coriandrum sativum*. The phytochemicals of *Anisomeles indica* of more or less similar pattern have been studied by Ulhe and Narkhede (2013) [24] and Kavitha *et al*., (2012) [25]. The phytochemicals of Terminalia bellerica have been thoroughly studied by Disha *et al*., (2014) and Nithya *et al.*, (2014) [26, 27]. Devendran and Balasubramanian (2011) [28] have screened the phytochemicals from *Ocimum sanctum*. Similar phytochemicals in aqueous ethanolic extract of *Ocimum sanctum* have also been studied by Bishnu *et al.*, (2011) [29]. Phytochemicals of *Azadirachta indica* and their antimicrobial activities have been studied by Susmitha *et al*., (2013) and Imran Khan *et al*., (2010) [30, 31]. Zemali *et al*., (2013) [32] have studied the phytochemicals of *Solanum nigrum* and also confirmed the presence of alkaloids, saponins, tannins, glycosides, coumarins, terpenoids, flavonoids and volatile oils. The phytochemicals and their antimicrobial activities of Citrus sinensis of similar characters have also been studied by Ehigbai *et al*., (2016) and Ashok Kumar *et al.*, (2011) [33, 34]. Plant derived extracts contain several biologically active compounds, many of which possess potent antimicrobial properties (M. Kumaraswamy et al. 2011) [35].

Conclusion

From the results it is evident that Ethanolic extracts of all the ten medicinal plants contained a significant amount of phytochemicals viz. alkaloid, flavonoids, phenolic, saponins and tannin. The amount of flavonoids was maximum in comparison to other phytochemicals in all the ten medicinal plants. Further evaluation of the active phytochemicals of these plants is required for definite conclusion of the bioactive compounds contributing to the antifungal and antiyeast activity. The nature of these active components is not very clear. Hence these compounds need further thorough investigation simultaneously and more studies are essential to purify and identify these compounds.

References

1. Vigers AJ, Roberts WK, Selitrennikoff CP. A new family of plant antifungal proteins, Molecular Plant and Microbe Interactions, 1991;4:315.
2. Vagi E, Simandi B, Suhajda A, Hethelyi E. Essential oil composition and antimicrobial activity of Origanum majorana L. extracts obtained with ethyl alcohol and supercritical carbon dioxide. Food Res. Int., 2005;38:51-57.
3. Nwaopara A, Anibeze C, Akpuaka F, Nwaopara S. Antimicrobial Potentials of Yaji-Spices: The Constituents of a Complex Nigerian Suya Meat Sauce Inducing Histological Investigations. The Internet Journal of Alternative Medicine. 2009, 6(2).
4. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews 1999;10:564-582.
5. Bowles DJ. Defense related proteins in higher plants Annual Review in Biochemistry, 1990;59:873.
6. Khan UA, Rahman H, Niaz, Qasim M, Khan, *Tayyaba et al.* Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. *Eur*. 1. *Microbiol. Immunol*. 2013;3:272-274. Doi: 10.1556/ Eu1Ml.3.2013.4.6.
7. Talib WH, Mahasneh AM. Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine. Molecules 2010;15:1811-1824.
8. Gould GW. New methods for food preservation. Blackie Academic & Professional, London., 1996b.
9. Smid EJ, Gorris LGM. Natural antimicrobials for food preservation. In M. S. Rahman (Ed.), Handbook of food preservation. New York: Marcel Dekker., 1999, 285-308.
10. Nakatani N. Antioxidative and antimicrobial constituents of herbs and spices In: Spices, Herbs, and edible Fungi (Ed.) G. Charalambous, Elsevier Science, New York, 1994, 251-271.
11. Yayé YG, Ackah JAAB, Kra AKM, Djaman AJ. Antifungal activity of different extracts of Terminalia mantaly H. Perrier on the in vitro growth of Aspergillus fumigatus. European Journal of Sciences Resources. 2012;82:132-138.
12. Harborne JB. Phytochemicals Methods.Chapman and Hall Ltd, London, 1973, 49-188.
13. Swain T, Tannins, Lignins. In: Resenthal, G. A. Jansen, D. H. (ed.). Herbovores, their interaction with secondary plant metabolites, Academic Press, New York, 1979, 657-682.
14. Brunner JH. Direct Spectrophotometer Determination of Saponin, Analytical Chemistry, Scientific Research, An Academic Publisher, 1984;34:1314-1326.
15. Folin O, Ciocalteu V. On Tyrosine and Tryptophane Determinations in Proteins. Journal of Biological Chemistry, 1927;73:627-650.
16. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Analaysis, 2002;10:178-182.
17. Emmanuel Ola Oshomoh, Macdonald Idu, Osayamen Collins Udinyiwe: Phytochemical Screening and Antimicrobial Sensitivity of Clove Flower (Syzygium aromaticum, L.Merril and Perry) Bud on Dental Pathogens, *IJPPR*, 2015;3(2):1-13.
18. Rashi Bankar, Ajai Kumar and Sadhna Puri: Phytochemical constituent of Syzygium aromaticum L, International Journal of Current Research, 2011; 3(7):215-217.
19. Umesh Kumar, Brajesh Kumar, Anil Bhandari and Y.Kumar: Phytochemical investigation and comparison of Antimicrobial screening of Clove and Cardamom, *IJPSR*, 2010;1(12):138-147.
20. Nirmala Paul, Sowmitra Kumar Sen, Mohammad Faisal Bin Karim, Nahid Hasan, Abddullah Faruque: Phytochemical and Biological investigations of *Coriandrum sativum* (Cilantro) leaves, *IJIPSR*, 2013;1(1):170-184.
21. Nimish Pathak l, Dr. Sanjay Kasture B, Nayna Bhatt M. Phytochemical screening of Coriander sativum Linn, International Journal of Pharmaceutical Sciences Review and Research, 2011;9(2):159-163.
22. Arun Thangavel, Senthilkumar Balakrishnan, Vijyalakshmi, Senbagam Duraisamy: Phytochemical screening, Gas Chromatography- Mass Spectrometry (GC-MS) analysis of Phytochemical constituents and Antibacterial activity of Coriandrum sativum (L), International, Journal of Pharmacy and Pharmaceutical Sciences, 2015;7(9):153-159.
23. Sasi Kumar R, Balasubramanian P, Govindaraj P, Krishnaveni T. Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of Coriandrum sativum L. roots (Coriander), Journal of Pharmacognosy and Phytochemistry, 2014;2(6):74-78.
24. Ulhe SK, Narkhede: Histological and Phytochemical studies on aromatic plant, *Anisomeles indica* (L.) of family *Lamiaceae* (M.S) India, Int J. of Life Sciences, 2013;1(4):270-272.
25. Kavitha T, Nelson R, Thenmozhi R, Priya E. Antimicrobial activity and Phytochemical analysis of *Anisomeles malabarica* (L) R.BR, J. Microbiol. Biotech. Res, 2012;2(1):1-5.
26. Disha Menpara, Dishant Desai, Sumitra Chanda: Pharmacognostic, phytochemical, physicochemical and fluorescence analysis of *Terminalia bellerica* leaf and stem, World Journal of Pharmaceutical Sciences, 2014;2(4):390-396.
27. Nithya Devi P, Kaleeswari S, Poonkothai M. Antimicrobial activity and phytochemical analysis of fruit extracts of *Terminalia bellerica*, Int J Pharm Pharm Sci, 2014;6(5):639-642.
28. Devendran G, Balasubramanian U. Qualitative phytochemical screening and GC-MS analysis of *Ocimum sanctum L.* leaves, Asian Journal of Plant Science and research, 2011;1(4):44-48.
29. Bishnu Joshi, Govind Prasad Sah, Buddha Bahadur Basnet, Megh Raj Bhatt, Dinita Sharma, Krishna Subedi,

et al. Journal of Microbiology and Antimicrobials, 2011;3(1):1-7.
30. Susmitha S, Vidyamol KK, Rangasnayaki P, Vijayaragavan R. Phytochemical extraction and antimicrobial properties of *Azadirachta indica* (Neem), Global Journal of Pharmacology, 2013;7(3):316-320.
31. Imran Khan, Surya Rao Srikakolupu, Surekha Darsipudi, Srujana Divya Gotteti, Hemasundara Amaranadh Ch Phytochemical studies and screening og leaf extracts of *Azadirachta indica* for its anti-microbial activity against dental pathogens, Archives of Applied Science Research, 2010;2(2):246-250.
32. Zemali Djaafar, Ouahrani M, Ridha. Phytochemical study of selected medicinal plants, *Solanum nigrum*, the Algerian Desert, International Letters of Chemistry, Physics and Astronomy, 2013;20:25-30.
33. Ehigbai I, Oikeh, Ehimwenma S, Omoregie, Faith E, Oviasogie, *et al*. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates, Food & Science Nutrition, 2016;4(1):103-109.
34. Ashok Kumar, Narayani KM, Subanthini A, Jaykumar M. Antimicrobial activity and phytochemical analysis of Citrus fruit peels-Utilization of fruit waste, International Journal of Engineering Science and Technology, 2011;3(6):5414-5421.
35. Kumara Swamy M, Pokharen N, Dahal S, Anuradha M. Phytochemical and antimicrobial studies of leaf extract of Euphorbia neriifolia, J. Med. Plants Res., 2011;5:5785-5788.