TBR
Trends in Biotechnology Research
An International Peer-Reviewed Journal
Online and Print Version
www.sciencejournal.in
DAMA INTERNATIONAL

PHYTOCONSTITUENTS OF A VALUABLE AYURVEDIC MEDICINAL HERB *CENTELLA ASIATICA* (L.) URB.

Sanjay R. Biradar*, Bhagyashri D. Rachetti* and Suryawanshi V.S.**
*Department of Botany, Tissue Culture Research Center, Shri Chhatrapati Shivaji College Omerga, Dist. Osmanabad, (MS), India.
**Department of chemistry, Shri Chhatrapati Shivaji College Omerga, Dist. Osmanabad, (MS), India.
(E-mail:sanjaybiradar2006@rediffmail.com)

ABSTRACT
Present study deals with the qualitative analysis of ethanolic extract of roots, stem & leaves & petiole of *Centella asiatica* (L.) Urb**.** In which we analyze 23 Phytochemicals which are use full for controlling the diseases in Human beings. *Centella asiatica* known as Brahmi, Indian Pennywort and Mandookaparni is a small herbaceous annual plant of the family Apiaceae, It is used in traditional medicine for the treatment of various ailments. *C.asiatica* is a native of India whichs creeper found in wetlands of most of the Sri Lanka, Northern Australia, Indonesia, Iran, Malaysia and other parts of Asian countries. The whole plant or the fresh leaves of the plant are widely used in ayurvedic preparations.The aim of the present study is to investigate the presence or absence of phytochemicals and to determine the Flavonoids, Alkaloids, Steroids, Proteins, Carbohydrates, Tannin, Amides, Terpenoides, Amines, Phenol, Test for Unsaturation, Carboxylic acid, Test for NH_2, Nitrogen, Sulphur, Halogen, Starch, Saponin, Ascorbic acid, Glycosides, Reducing Sugar and Triterpenoids contents of the selected medicinal plants.

KEYWORDS: Phytoconstituents; ayurvedic medicinal herb; *Centella asiatica* (L.) Urb; Qualitative analysis

INTRODUCTION

Centella asiatica known as Brahmi, Indian Pennywort and Mandookaparni is a small herbaceous annual plant of the family Apiaceae. Centella asiatica L. has been used as a medicinal herb for thousands of years in India, China, Sri lanka, Nepal and Madagascar. *Centella asiatica* is one of the chief herbs for treating skin problems, healing wounds, revitalizing nerves and brain cells, hence it is primarily known as a "Brain food" in India. (Singh, 2010). Vegetation shown in figure 1.

Figure 1. Photograph showing vegetation of *Centella asiatica* (L.) Urb.

It has been used for wound healing, better blood circulation, memory enhancement, anti-carcinogenic, Apoptosis Induction of *Centella asiatica* on Human Breast Cancer Cells (Suboj Babykutty, 2009) and also has been used for respiratory ailments, detoxifying the body, treatment of skin disorders (such as psoriasis and eczema), revitalizing connective tissue, burn and scar treatment, clearing up skin infections, slimming and edema, arthritis, rheumatism, treatment of liver and kidneys, periodontal disease, strengthening of veins (varicose veins), blood purifier, high blood pressure, sedative, anti-stress, anti-anxiety, an aphrodisiac, immune booster, anabolic etc. (Wing, 2007)

Phytochemicals are the natural bioactive compounds found in plants. These phytochemicals work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions (Koche, 2010). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, steroid, terpenoid, carbohydrate and phenolic compounds (Pascaline, 2011). The medicinal value of these plants lies in some chemical substances that have definite physiological processes in the human body. The most important of these bioactive constituents of plants are alkaloids, terpenoids, carbohydrates and proteins compounds **(**Dharmendra Singh,2012).

The primary constituents of *C. asiatica* is the triterpenic fractions which showed wide range of defensive and therapeutic effects, most prominently influencing of collagen production and deposition in wound healing. Titrated Extract of *Centella asiatica* (TECA) is used to treat several microcirculatory problems (Dipankar Chandra Roy, 2013).

MATERIALS AND METHODS

1. Collection of plant material

The fresh parts of *Centella asiatica* (L.) Urb. were collected in flowering period from Amrutkund Tq.Basavkalyan, Dist. Bidar near Maharashtra-Karnataka border on 9th Feb.2013. The plant material were properly washed with tap water and then rinsed with distilled water, dried in oven at 60^{0}C until plant parts became well dried for grinding. After drying, the plant materials were ground well into fine powder.

2. Preparation of ethanolic extracts from different plant parts (Roots, Stem, Leaf, Petiole)

For preparation of ethanolic extract, a modified method of **Abdulrahman (2004)**was used. The fresh parts of the plant were dried in oven and ground to fine powder with mechanical grinder. Ten gram of each plant parts was then macerated in 100 ml of absolute ethanol for 48 hr. & properly covered with aluminium foil & labeled. After 48 hrs of extraction, each extract was filtered through Whatman's filter paper no.1 separately. The filtrate was evaporated to dryness at room temperature & store at 5^{0}C in refrigerator.

3. Qualitative analysis

3.1. Test for Alkaloids

Ethanolic extract was warmed with 2% H2SO4 for two minutes. It is filtered and few drops of reagents were added and indicated the presence of alkaloids.

a. Mayer's reagent-A creamy- white colored precipitation positive.
b. Wagner's reagent-A reddish-brown precipitation positive.
c. Picric Acid (1%)-A yellow precipitation positive.

3.2. Test for Steroids Terpenoid and Triterpenoids:

a) Liebermann Burchard test - Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids terpenoid and triterpenoids respectively.

b) Salkowski Test

The extract was mixed with 2ml of chloroform and concentrate H2SO4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of steroids terpenoid and triterpenoids respectively.

3.3. Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

3.4. Test for phenols and tannins

Crude extract was mixed with 2ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols and tannins.

3.5. Test for Flavonoids

A small quantity of the extracts is heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture is filtered differently and the filtrates are used for the following test.

a) Ammonium Test

The filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed at ammonia layer. This indicates the presence of the flavonoid.

b) Aluminum Chloride Test

The filtrates were shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow color. It indicated the presence of flavonoid and diluted NaOH and HCl was added. A yellow solution that turns colorless indicated positive.

3.6. Test for Carbohydrate:

Benedict's test – Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

3.7. Test for Glycosides

Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

3.8. Test for proteins

Millon's test

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

3.9. Test for Free Amino Acids:

Ninhydrin Test – Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation ofpurple color suggesting the presence of free amino acids.

3.10. Test for Vitamin C:

DNPH Test – Test solution was treated with Dinitrophenylhydrazine dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin C.

Qualitative phytochemical analyses were done using the procedures of Kokate, 1994 for Alkaloids, carbohydrates, tannins, phenols, flavonoids, and saponins. For Carboxylic acid, test for NH_2, Nitrogen, Sulphur, Halogen, Amides, test for Unsaturation, test for Aromaticity. (Clarke, 2007) . Test for Starch, Steroids, Proteins, Glycosides, Reducing sugar and Ascorbic Acid (Arun Sethi, 2003) test for Amino acid (Pratibha Devi 2003) Liebermann - Burchard's test for Triterpenoides, Terpenoides were qualitatively analyzed.

RESULTS AND DISCUSSION

Qualitative analysis of ethanolic extract of root, stem, leaf and petiole of ***Centella asiatica*** **(L.) Urb.** in which we have analyzed 23 phytochemical tests. This report has also revealed that the ethanolic extract used for qualitative phytochemical analysis which gives better results. *C. asiatica* is a source of Flavonoids, Steroids, Proteins, Carbohydrates, Tannin, Amides, Terpenoides, Amines, Phenol, Carboxylic acid, Starch, Saponin, Ascorbic acid, Glycosides, Reducing Sugar, Triterpenoids, & Amino acids,etc. (Table 1).

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh,2007). They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antihelminthic (Dama and Jadhav, 1998; Dama *et al.*, 1998; Dama, 2002; Dama and Kirdak 2002), antiinflammation (Poul *et al.*, 1999), antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han,2007).

Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown,1998; Krings, 2001). The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation (Just,1998; Jawale et al., 2010; Jawale and Dama 2010a; 2010b; Jawale *et al.*, 2012**).** Saponins has the property of precipitating and coagulating red blood cells. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity. The preliminary phytochemical tests are helpful in finding chemical constituents in the plant

material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical Compound.

Table 1: Distribution of various phytochemical analysis in different parts of *Centella asiatica* (L.) Urb. Plant.
Preliminary (Qualitative) phytochemical analysis of Ethanolic extract of *Centella asiatica* (L.) Urb..

Sr. No	Compound / Part	Root	Stem	Leaf	Petiole
1.	Alkaloids	_	+	+	_
2.	Amides	+	+	+	+
3.	Amines	+	+	+	+
4.	Ascorbic acid	_	_	+	_
5.	Carbohydrates	+	+	+	+
6.	Carboxylic acid	+	+	+	+
7.	Flavonoids	+	+	+	+
8.	Glycosides	+	+	+	+
9.	Phenol	+	+	+	+
10.	Proteins	+	+	+	+
11.	Reducing Sugar	+	+	+	+
12.	Saponin	_	+	+	+
13.	Starch	+	+	+	+
14.	Steroids	+	+	+	+
15.	Tannin	+	+	+	+
16.	Terpenoides	+	+	+	+
17.	Test for amino acid	+	+	+	+
18.	Test for Aromaticity	+	+	+	+
19.	Test for Halogen	_	_	_	_
20.	Test for Nitrogen	+	+	+	+
21.	Test for Sulphur	_	_	_	_
22.	Test for Unsaturation	_	_	_	_
23.	Triterpenoids	+	+	+	+

+ = indicates presence of bioactive compound, - = indicates absence of bioactive compound

CONCLUSION

In the present investigation, qualitative phytochemical analysis of different parts of *C.asiatica* gives better results but it is observed that alkaloids, ascorbic acid, saponin are absent in root, where as ascorbic acid is absent in stem, alkaloids and ascorbic acid are absent in petiole too. Halogen, sulpher and Unsaturation (C=C) are absent in all parts of *C.asiatica*. As C.asiatica has extensive scope in pharmacology. So, it needs the further study of *C.asiatica*.

ACKNOWLEDGEMENT

Author Dr.Sanjay Biradar, Principal Iinvestigator is grateful thanks to UGC– New Delhi for sanctioned the Major Research Project [F.No. 41-479/2012(SR)] and also thankful to Principal of Shri Chatrapati Shivaji College, Omerga, Dist. Osmanabad, (M.S.), India for providing all necessary facilities and encouragement for the present research work.

REFERANCS

Abdulrahman F., Inyang S.I., Abbah J., Binda L., Amos S., Gamaniel K. (2004). Effect of aqueous leaf extracts of Irvingia gabonesis on gastrointestinal tracts of rodents. *India J. Exp. Biol.* 42:787-791.

Arun Sethi (2003). Lab Experiments in Organic Chemistry, New Age International (P) Limited, Publisher, ISBN 81-224-1491-5.

Brown J.E. and Rice-Evans C.A. (1998). Luteolin rich artichoke extract protects low density lipoprotein from oxidation in vitro. *Free Radical Res.* 29: 247-255.

Clarke Hans Thacher (2007). A Handbook of Organic Analysis, IV Edn., CBS Publishers, New Delhi.

Dama L.B and Jadhav B.V. (1998). Cestocidal activity of Vidhang fruit extract. *Riv. di Parassitol.* 15(3): 249-252.

Dama L.B., Poul B.N. and Jadhav B.V. (1998). Antimicrobial activity of Napthoquinonic compounds. *J. Ecotoxicol.Environ. Monit.* 8: 213-215.

Dama L.B. (2002). Effect of naturally occurring napthoquinones on root- knot nematode *Meloidogyne* spp. *Indian Phytopathol.* 55 (1): 67-69 (2002).

Dama L.B and Kirdak R.V. (2002). Effect of Vidhang seed extract against *Ascaridia galli* in naturally infected fowls (*Gallus domesticus*). *J. Parasitic Dis.* 26: 48-49.

Dharmendra Singh., Poonam Singh., Abhishek Gupta., Shikha Solanki., Ekta Sharma (2012). *Rajeev Nema1 Int. J. Life Sci. Med. Res.* LSMR, LSMR Vol.2 No.1 2012 PP.5-7 www.jlsmr.org ,C World Academic Publishing.

Dipankar Chandra Roy., Shital Kumar Barman and Md. Munan Shaik (2013). Current Updates on *Centella asiatica*: Phytochemistry, Pharmacology and Traditional Uses. *Medicinal Plant Res*. 3: 20-36.

Han, X., Shen, T., Lou, H. (2007). Dietry polyphenols and their biological significance. Int. J. Mol. Sci., : 950-988.

Jawale C.S., Kirdak R.V. and Dama L.B. (2010). Larvicidal Activity of *Cestrum nocturnum* (Solanaceae: Solanales) on Aedes *aegypti*. *Bangladesh J. Pharmacol.* 5: 39-40.

Jawale C.S. and Dama L.B. (2010a). Hematological changes in the fresh water fish, *Cyprinus carpio* exposed to sub-lethal concentration of piscicidal compounds from *Cestrum* species (Family : *Solanaceae*). *Nat. J. Life Sci.* 7(1): 81-84.

Jawale C.S., Dama L.B. (2010). Insecticidal potential of *Cestrum* sp. (Solanaceae: Solanales) against *Tribolium castaneum* and *Tribolium confusum* (Herbst) (Coleoptera- Tenebrionidae). *Deccan Curr. Sci.* 3(2): 155-161.

Jawale C. S., Dama L. B., Pawar Kishor, Dama S.B. and Shaikh Yasmeen (2012). Cestrum nocturnum (L) A Prospective Piscicide for Control of Predatory Fish Channa punctatus (Bloch.). *Trends Fisheries Res.* 1(1): 14-17.

Just M.J., Recio M.C., Giner R.M., Cueller M.U., Manez S., Billia A.R., Rios J.L. (1998). Antiinflammatory activity of unusual lupine saponins from *Bupleurum fruticescens. Planta Medica.* 64: 404-407.

Koche D. R. and Shirsat S Imran.(2010). Phytochemical screening of eight traditionally used ethnomedicinal plants from akola district (ms) India. *Int. J. Pharma Bio Sci.* 1(4).

Kokate, C. K. (1994). Practical Pharmacognosy, 3rd Ed, Vallabh Prakashan, NewDelhi

Krings U., Berger R.G. (2001). Antioxidant activity of roasted foods. *Food Chem.* 72: 223-229.

Pascaline, J.,M Charles, Lukhoba C. (2011) . Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi district Kenya. *J. Anim. Plant Sci.* 9(3): 1201- 1210.

Poul B.N., Mukadam D.S. and Dama L.B. (1999). Anti-inflammatory activity of *Plumbago zylanica*. *Fronteries Bot.* 1: 91-93.

Pratibha Devi (2003). Principles and Methods of Plant Molecular Biology, Biochemistry and Genetics. *Agrobios.*

Singh S. Gautam A. and Sharma A. (2010). Centella asiatica L.: a plant with immense medicinal potential but threatened. *Int. J. Pharmaceutical Sci. Review Res.* 4(2): Article 003.

Singh, R., Singh, S.K., Arora, S. (2007). Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis. *A. Cunn. Fod Chem. Toxicol.* 45: 1216-1223.

Suboj Babykutty, Jose Padikkala, Priya Prasanna Sathiadevan, Vinod Vijayakurup, Thasni Karedath Abdul Azis,Priya Srinivas, and Srinivas Gopala, Afr J. (2009),Tradit Complement Altern Med. 6(1): 9–16.Published online 2009 October 25.PMCID: PMC2816528.

Wing-Y Li., Chan Shun-Wan., Guo De-Jian., Peter., Yu Hoi-Fu. (2007). Correlation between antioxidative power and anticancer activity in herbs from traditional Chinese medicine formulae with anticancer therapeutic effect. *Pharmaceutical Biol*. 45:541–546.

