International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **2(10),** 18-21, October **(2013)** *Int. Res. J. Biological Sci.*

Phytochemical screening and Antibacterial Activity of Western Region wild leaf *Colocasia esculenta*

Nakade Dhanraj B[1]., Mahesh S. Kadam[2], Kiran N. Patil[3] and Vinayak S. Mane[2]
[1]Department of Microbiology, Govt. Rajaram College, Kolhapur-416004, MS, INDIA
[2]Department of Microbiology, Dr. Ghali College, Gadhinglaj-416502, Dist. Kolhapur, MS, INDIA
[3]Department of Chemistry, Dr. Ghali College, Gadhinglaj-416502, Dist. Kolhapur, MS, INDIA

Available online at: www.isca.in, www.isca.me
Received 19th June 2013, revised 8th July 2013, accepted 8th August 2013

Abstract

The present study aims at evaluating the antibacterial activity and phytochemical screening of Colocasia esculenta. It is an important medicinal plant in India which is used in traditional medicine. The leaves are rich in vitamins and minerals. Leaves of Colocasiaesculenta were extracted with organic solvent like ethyl acetate and its biological activity against antibacterial strain was checked for 100 ppm concentration. Leaf juice of this plant is applied over scorpion sting or in snake bite as well as it is used in food poisoning of plant origin.

Keywords: Colocasia esculenta Linn, extracts and phytochemistry, antibacterial properties, pathogens, disc diffusion method.

Introduction

Colocasia is a genus of 25 or more species of flowering plants of family Araceae, native to tropical region and southeastern Asia. Common names include Elephant-ear, Cocoyam, Chembu, and Eddoe. Elephant-ear and Cocoyam are also used for some other large-leaved genera in the Araceae[1]. It is thought to be the oldest cultivated plant in the world, having been cultivated in Asia for more than ten thousand years. *Colocasia esculenta* is herbaceous perennial plant belonging to the Araceae family however the leaves are also used as leafy vegetables. Leaf juice of this plant is applied over scorpion sting or in snake bite as well as it is used in food poisoning of plant origin[2].

The large green leaves are often described as 'elephant ear'and they can reach up to 1-2 m high during growth. The starchy and tuberous root is the main edible part of the crop, however the leaves are also used as the leafy vegetable. *Colocasiaesculenta*leaves have been reported to be rich innutrients including minerals and vitamins such as calcium, phosphorous, iron, vitamin C, thiamine, riboflavin and niacin[3,]. Among various edible aroids commercially cultivated in India, *Colocasiaesculenta* assume note-worthy dietary significance having multiple uses in the form of various culinary preparations of its corm and edible stem. Fresh edible leaves of *Colocasiaesculenta*form rich source of protein, ascorbic acid, dietary fibre and some nutritionally important minerals[4].

Material and Methods

Sample Collection: The fresh parts of healthy leaves of *Colocasiaesculenta was* collected from Bellad corner of Gadhinglag, Tal-Gadhinglag, Dist-Kolhapur, Maharashtrain the month of November – December 2012 with proper identification. The leaves were washed with tap water then by distilled water and finally dried. Dried sample was grinded into fine powder by the help of a grinder.

Preparation of Plant Extract: Plant material was separated into two different parts such as tuber and leaves. Only leaves were fine powered into grinder. Twenty gram of each powdered plant material was extracted separately at room temperature using various solvents namely methanol, water, ethylacetate with gentle stirring for 24 hrs. The filtered solvent was concentrated in water bath for 6 hrs[5-7].

Phytochemical Screening: i. **Detection of alkaloids**: Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate. ii. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates. iii. Detection of phenols: Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols. iv. Detection of Tannin: The 4 ml extract was treated with 4 ml $FeCl_3$ after which formation of green colour was taken as positive for tannin. v. Detection of flavonoids: Alkaline Reagent Test: Extracts were treated with few drops of 10 % sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of

flavonoids. vi. Detection of saponins: Froth Test: Extracts were diluted with distilled water up to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins. Foam Test: The 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins. vii. Detection of Steroids: Extracts was treated with 3-4 drops of chloroform and a drop of acetic acid were heated it for some time and then concentrated Sulphuric acid was added which gives orange colour indicates presence of steroids. viii. Detection of Quin one: Extracts were treated with conc. HCl which gives green colour indicates presence of quinones. ix. Detection of cellulose: Extracts were treated with iodine and by addition of 2 to 3 drops of concentrated sulphuric acid development of brown colour, was taken as presence of cellulose. x. Detection of Terpenoids: Extracts were treated with 2ml chloroform followed by conc. sulphuric acid which gives light orange colour, indicates presence of terpenoids. xi. Detection of glycosides: Extracts were mixed with 2ml glacial acetic acid and a drop of $FeCl_3$ and then 1ml sulphuric acid which gives brown colour indicatespresence ofglycosides.

Antibacterial Susceptibility Assay: Antibacterial susceptibility assay was carried out by disc diffusion method[8-12]. Where in six bacterial pathogens were used. *E.coli, Staphylococcusaureus, Salmonella typhi, Bacillussubtilis, Proteusvulgaris, Klebsiellapneumoniae*. All bacterial cultures were spread into the separate plate. 6mm diameter filter paper disc was soaked in plant extract and dried at 50°C. This disc was placed on the surface medium which is having bacterial strain;plates were kept for diffusion at 4°C and were incubated at 37°C for 24 hours. After 24hours zone of inhibition was measured for detecting antibacterial activity.

Interpretation of results: Results were interpretated as per clinical and laboratory standards institute (CSLI) guidelines[13-15].

Results and Discussion

Table 1 indicates phytochemical analysis of leaf extracts. Results indicated that leaf extract contains phenols, tannin, saponins, steroids, quinine, trepenoids, glycosides, alkaloids except flavonoids.

Table 2 indicates antibacterial activity of various extracts, of all these ethyl acetate extract was found to be most effective against *Pseudomonas aeruginosa*, which correlated with results of Negi and Dave[16].

From figure 1 it is indicated that aqueous extract of leaf was found to be effective against Klebsiella pneumonia and methanolic extract was found to be effective against *Salmonella typhi* and *Klebsiella pneumonia*. Govindrajan *et al* showed various solvent extracts of leaves exhibited inhibitory property against *Staphylococcus aureus, Staph. Epidermis, Bacillus cereus, Streptococcus fecalis*[17].

Table-1
Phytochemical Analysis of leaf extract of *Colocasiaesculenta*

Sr.No.	Secondary Metabolite	Ethyl Acetate extract
1.	Phenols: FeCl3 Test	+
2.	Tannin : Fecl3 Test	+
3.	FlavonoidAlkaline reagent test	-
4.	Saponins: Foam Test	+
5.	Steroid	+
6.	Quinone	+
7.	Cellulose	+
8.	Terpenoids	+
9	Glycosides	+
10	Alkaloids	+

Note: (+) = Present, (-) = Absent

Table-2
Antibacterial Susceptibility Assay of *Colocasiaesculenta* Leaves Extracts

Sr. No.	Pathogens	E.A.E.	M.E.	A.E.
1.	*Salmonella typhi*	6 mm	10 mm	7 mm
2.	*Klebsiellapneumoniae*	8 mm	10 mm	11 mm
3.	*Pseudomonas aeruginosa*	12 mm	-------	------
4.	*Salmonella paratyphi*	------	------	------
5.	*Saphylococcusaureus*	-------	-------	------
6.	*Bacillus subtilis*	8 mm	6 mm	9 mm
7.	*Proteus vulgaris*	--------	10 mm	-------
8.	*E-coli*	-------	8 mm	7 mm

E.A.E.-Ethyl Acetate Extract, M.E.-MethanolicExtract, A.E.-Aqueous Extract, Note: (+) = Present, (-) = Absent

Quite number of plant extracts have been proposed by various researchers, Aqil et al, proposed Acetone fraction of Rhizomes which has inhibitory activity against Methicillin resistant Staph. Aureus[18]. Durodola reported antibacterial activity of crude extract against Helicobacter pylori[19]. Our results showed that the leaves extract of *Colocasis esculenta* is effective against *Salmonella typhi, Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis, Proteus vulgaris and E.coli,* this indicated that the leaves extract can be used for treatment of Typhoid, Pneumonia, Otitis, Urinary tract infection and Diarrhea.

Figure 2 The photographs showing the antibiogram of *Colocasiaesculenta* leaves extracts in various solvents performed against available pathogens

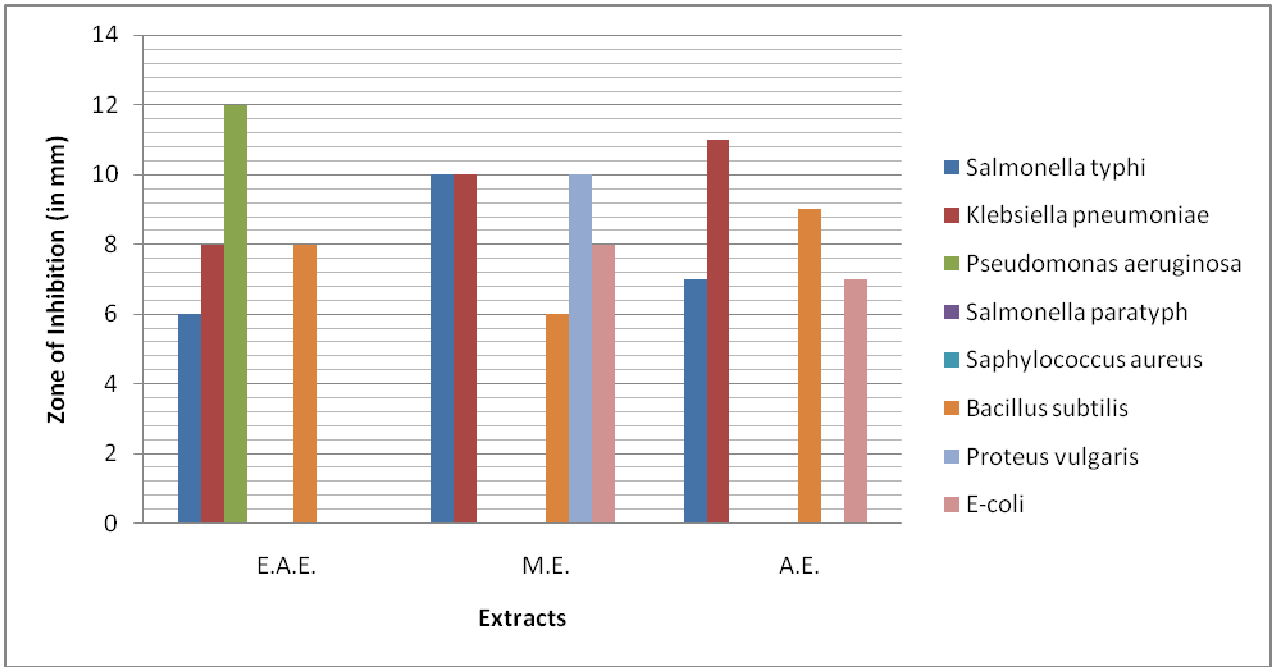

Figure-1
Comparative analysis of *Colocasia esculenta* leaves extracts against chosen Pathogens

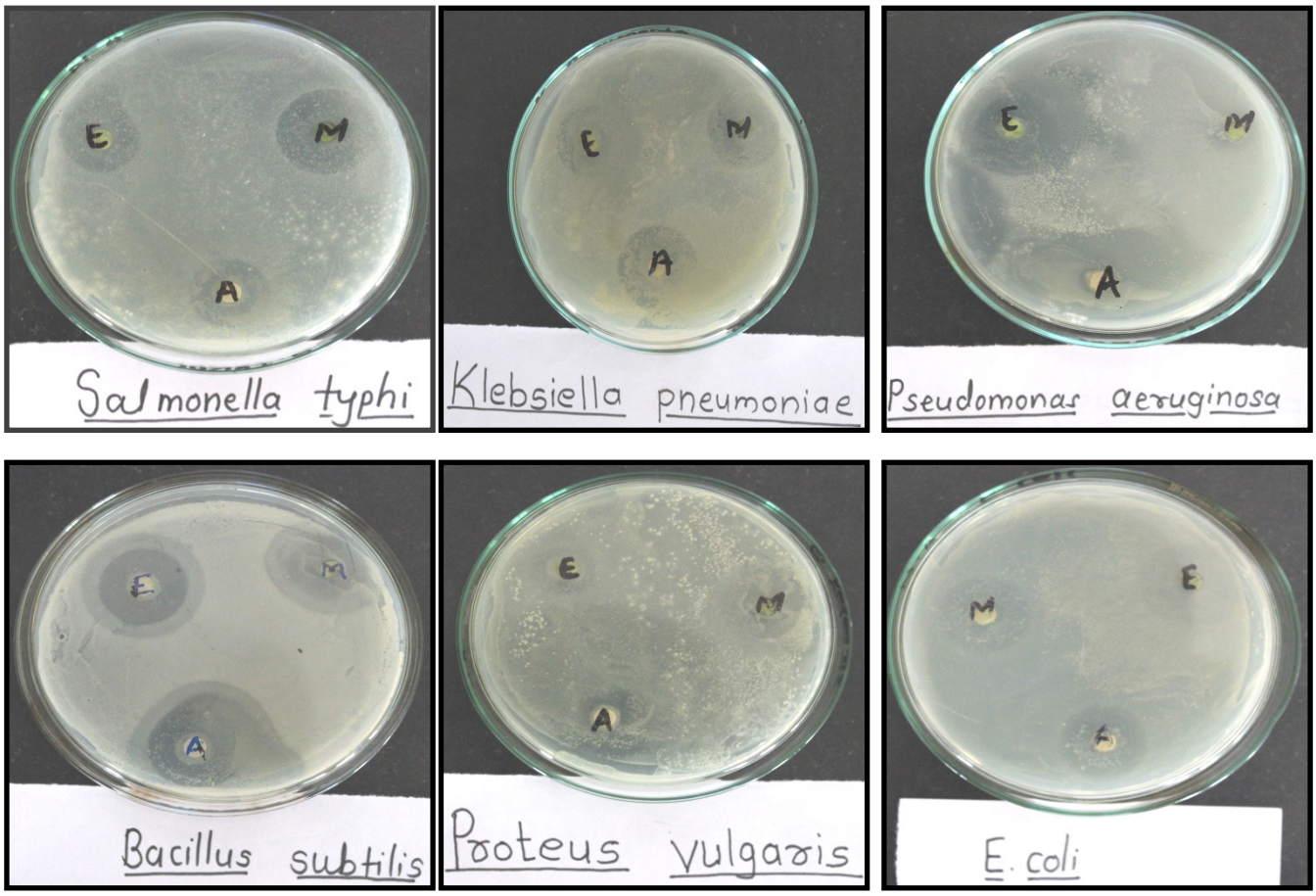

Figure-2
Antibiogram of *Colocasiaesculenta* leaves extracts in various solvents performed against available pathogens

Conclusion

It can be concluded that ethyl acetate leaves extract contain more number of phytochemicals. Antibacterial susceptibility assay indicated that the ethyl acetate extract showed the highest activity against pathogenic bacterial strains we recommend it in treatment of Typhoid, Pneumonia, Otitis, Urinary tract infection and Diarrhoea infections.

Acknowledgement

We are verymuch thankful to Principal Rajaram college, Kolhapur and Principal, Dr. Ghali college, Gadhinglaj for allowing us to work in laboratories and other support.

References

1. www.wikipedia.org **(2013)**

2. Chandra Subash,SakalaniSarala and Sing Jaybardhan, *International Journal of Pharmacy,* **3(3),** 181-186, **(2012)**

3. Sharma P.C., Yelne M.B. and Dennis T.J., *Database on Medicinal Plants Used in Ayurveda*., 369-377 **(2001)**

4. Sahoo K.P., Pawan K. Kasera and Sher Mohammed, *Asian Journal of Plant Science and Research*, **2(6),** 650-652 **(2012)**

5. Bhagyashree R. Patil and Hussein M. Ageely, *International Journal of Advanced Biotechnology and Research,* **2(2)**, 296-304 **(2011)**

6. Tarun Agarwal, Rachana Singh, Amar Deep Shukla and Imran Waris, *Asian Journal of Plant Science and Research*, **2(1)**, 36-40 **(2012)**

7. Lewu M.N., Adebola P.O. and Afolayan A.J., *African Journal of Biotechnology*, **8(8)**, 1619-1622 **(2009)**

8. Prajapati A., Synthesis, Antimicrobial and Insecticidal Activity Studies of 5-Nitro N' -[Arylidenhydrazidomethyl Indole] 2-(Substituted Aryl) -3-(N'-Indolyl Acetamiddyl)-4- Oxothiazolidines, *Research Journal of Recent Sciences,* **1(ISC-2011),** 99-104 **(2012)**

9. Mangale S.M., Chonde S.G. and Raut P.D., Use of *Moringa Oleifera* (Drumstick) seed as Natural Absorbent and an Antimicrobial agent for Ground water Treatment, *Research Journal of Recent Sciences,* **1(3),** 31-40 **(2012)**

10. Lawrence Drew, A.L. Barry, Richard O'Toole, AND John C. Sherris, *Applied Microbiology*, **24(2),** 240-247 **(1972)**

11. Signe Ringertz, Margareta Rylander and Goran Kronyall, *Journal of Clinical Microbiology*, **29(8),** 1604-1609 **(1991)**

12. Khan J.A. and Tewari S., *Asian Journal of Plant Science and Research*, **1(1),** 22-30 **(2011)**

13. Bauer A.W., Kirby W.M., Sherris J.C. and Truck M., Antibiotic susceptibility testing by a standardized single disc method, *J. clin.path*., **45,** 493-496 **(1996)**

14. Clinical and Laboratory Standards Institute performance standards (CSLI) for antimicrobial discs tests: approved guidelines, **31(1) (2011)**

15. Anantnarayan R. and Paniker, Textbook of microbiology,8th edition, Orient longman, ltd., 186-307 **(2009)**

16. Negi B.S. and Dave B.P., Antimicrobial activity of Acacia and its phytochemical analysis, *Indian Journal of Microbiology,* 50, 369-374 **(2010)**

17. Govindrajan M.A, Jebanesan D., Reetha R., Asmath T., Pushpanathan and Samidurai K., Antibacterial activity of Acalypha indica, *Europian review for Medical and Pharmacological Sciences*, **12,** 299-302 **(2008)**

18. Aqil F., Ahma D.I. and Owais M., Evaluation of Anti-Methicillin Resistant Staph aureus activity and synergy of some bioactive plant extracts, *Biotechnology Journal*, **1,** 1093-1102 **(2006)**

19. Durodola J.I., Antibacterial property of crude extracts from a herbal wound healing remedy Ageratum conyzoides L. Planta Medicaa, **32,** 388-390 **(1977)**