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EFFECT OF ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *Acalypha indica* L.

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ABSTRACT

The aime of the study was to investigate the phytochemical and antimicrobial properties of *Acalypha indica* against bacteria *Escherichia coli, Staphylococcus aureus and* fungal *Candida albicans and Aspergillus flavus. The* results of this study clearly indicate that The phytochemical screening methanol extract of *Acalypha indica* leaf showed that the presence of alkaloids, steroids, saponins, flavonoids, terepenoids, phenolics, triterpenoids, carbohydrate, glycosides, and anthriquinone while tannin, phlobatannins and protein were absent. Aqueous extract of *Acalypha indica* leaf showed that the presence of protein, steroids, saponins, triterpenoids, phenolics, carbohydrate, anthriquinone, glycosides, flavonoids, terepenoids, while tannin, phlobatannins and alkaloids were absent. Significant amount of phytochemicals present. Histochemical study further confirmed the presence of phytochemicals. The results reveal that extract of *Acalypha indica* leaf was significantly effective against bacteria species *E. coli, St. aureus* and fungi species *C. albicans* and *A. flavus.*. The synergistic effect of plant extract against resistant bacteria and fungi leads to new choices for the treatment of infectious diseases. Overall, the *Acalypha indica* leaves are a rich source of phytochemicals and potential antimicrobial activity that can be important in infectious disease prevention and health preservation.

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INTRODUCTION

Human beings have used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1989). According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements, since they cannot afford the products of Western pharmaceutical industries (Salie *et al*., 1996), together with their side effects and lack of healthcare facilities (Griggs *et al*., 2001). Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine (Mann *et al*., 2008; Ammara *et al*., 2009). People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses (Van Wyk *et al*., 2000).

Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high

safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Tapsell *et al*., 2006). Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people.

The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from natural sources including plants. Plant and plant products play a wide range of antimicrobial properties. Keeping in view, the present study to investigate the phytochemical and antimicrobial properties of *Acalypha indica* against bacteria *Escherichia coli*, *Staphylococcus aureus* and fungal *Candida albicans* and *Aspergillus flavus*.

MATERIALS AND METHODS

Plant materials

The *Acalypha indica* leaves were collected in January 2017 from Thanjavur, Tamil Nadu, India.

Quanlitative analysis of phytochemicals

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984). Qualitative Analysis Of Vitamins analysed by the method of Pearson, (1976) and Patel, (2005).

Quantitative analysis of phytochemicals

Determination of total phenols by spectrophotometric method. Saponin determine by the method of Obadoni and Ochuko (2001). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994). Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956).

Histochemical Analysis

The powder of *Acalypha indica* was treated with specific chemicals and reagents. The treated plant powder further analysed in light microscope. The *Acalypha indica* treated with phloroglucinol and diluted HCl to gave red colour indicates lignin, treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids and treated with Dragant draft reagent gave brown colour indicates alkaloids. Plant powder treated with ferric chloride to gave Dark blue to black indicates the presence of tannin. Plant powder treated with Conc HCl to gave Dark Black indicates the presence of crystals. Plant powder treated with 5 drops of acetic anhydride and 5 drops of H_2SO_4 to gave Violet to Blue (or) Green colour indicates the presence of steroids. Plant powder treated with Toludine blue to gave Blue green/Red colour indicates the presence of polyphenol. Plant powder treated with Dinitrophenol hydrazine (few drops) to gave Orange colour indicates the presence of Terpenoids. Plant powder treated with H2SO4 (few drops) to gave Yellow colour indicates the presence of Saponin.

Determination of Antimicrobial Activity

Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al*., 2007) using plant extract. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Staphylococcus aureus*, *Escherichia coli* specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans* and *Aspergillus flavus*, were spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

RESULTS

Phytochemical analysis of *Acalypha indica*

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Acalypha indica* leaf investigated and summarized in Table-1 and fig- 2 and 3. The phytochemical screening methanol extract of *Acalypha indica* leaf showed that the presence of alkaloids, steroids, saponins, flavonoids, terepenoids, phenolics, triterpenoids, carbohydrate, glycosides and anthroquinone while tannin, phlobatannins and

protein were absent. Aqueous extract of *Acalypha indica* leaf showed that the presence of protein, steroids, saponins, triterpenoids, phenolics, carbohydrate, anthriquinone, glycosides, flavonoids, terepenoids, while tannin, phlobatannins and alkaloids were absent (Table-1and Figure-2).

Histochemical analysis

The use of histochemical characters in taxonomic conclusions is now a common practice. Table 2 and figure-3 represents histochemical studies of *Acalypha indica* leaf powder. This study further confirmed the presence of phytochemicals in *Acalypha indica* leaf.

Qualitative analysis of vitamins

In the present study vitamin C and E were present and represent in table 3 and figure- 4. Significant amount of Flavonoids (50mg/gm), terpenoids 10(mg/gm) and phenol (110mg/gm) and saponnin (30mg/gm).

Determination of Antimicrobial Activity

Plant extract of *Acalypha indica* was screened against *Escherichia coli* and *Staphylococcus aureus* species of bacteria and *Candida albicans* and *Aspergillus flavus* species of fungi were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Acalypha indica* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Figure -5 The inhibitory activities in culture media of the *Acalypha indica* reported in Table 4 were comparable with standard antimicrobiotic viz. chloromphenical and fluconazole.

Table- 1: Phytochemical screening of *Acalypha indica* leaf

S. No	Phytochemical analysis	70% methanol extract	100% Aqueous extract	Quantitative analysis (mg/gm)
1	Tannin	---	---	---
2	Phlobatannins	---	---	---
3	Saponin	+	+	30
4	Flavonoids	+	+	50
5	Steroids	+	++	---
6	Terepenoids	+	++	---
7	Triterpenoids	+	+	10
8	Alkaloids	+	---	---
9	Carbohydrate	+	+	---
10	Protein	---	+	---
11	Anthroquinone	+	+	---
12	Polyphenol	+	+	110
13	Glycoside	+	+	---

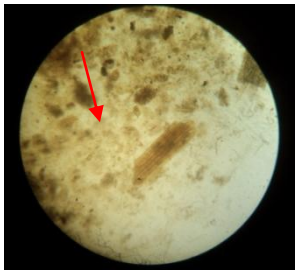
(+) Presence, (++) highly presence and (-) Absence

Table – 2: Histochemical studies of *Acalypha indica* leaf powder

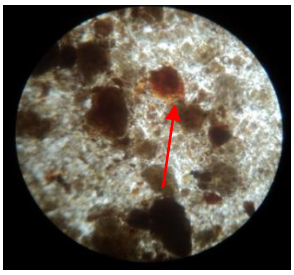
S. No.	Secondary metabolites	Observation	Result
1	Alkaloids	Reddish Brown	+
2	Tannin	Dark Blue to Black	+
3	Flavonoids	Yellow	+
4	Steroids	Violet to blue (or) Green	+
5	Polyphenol	Blue green/Red	+
6	Terpenoids	Orange	+
7	Saponin	Yellow	+

(+) Presence ; (-) Absence

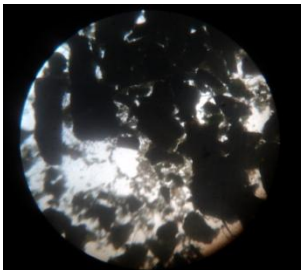
Figure-3: Histochemical studies of *Acalypha indica* leaf powder



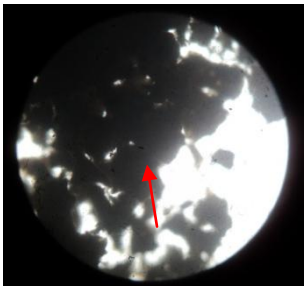
Flavonoids



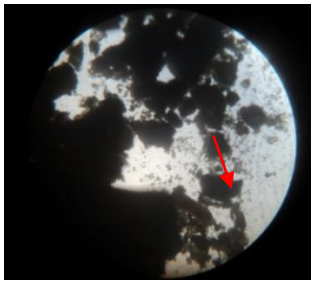
Alkaloids



Polyphenol

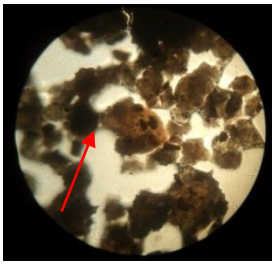


Tannin



Steroids

Terpenoids



Saponin

Table - 3 : Vitamin analysis of *Acalypha indica* leaf powder

S. No	Name of the Test	Results
1	Vitamin A	---
2	Vitamin C	+
3	Vitamin D	---
4	Vitamin E	+

(+) Presence ; (-) Absence

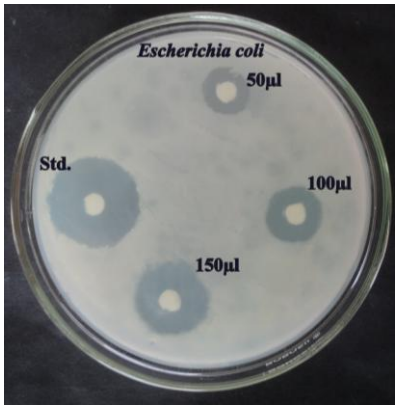
Table -4: Antimicrobial activities of *Acalypha indica*

Microbial Organism	50µl	100µl	150µl	Standard
Escherichia coli (mm)	9.70±0.26	8.70±0.46	9.00±0.56	9.70±0.78
Staphylococcus aureus (mm)	6.00±0.22	7.50±0.37	7.70±0.50	7.70±0.76
Candida albicans (mm)	6.70±0.18	8.20±0.34	7.00±0.46	7.20±0.75
Aspergillus flavus (mm)	8.50±0.11	7.70±0.25	10.50±0.43	10.70±0.70

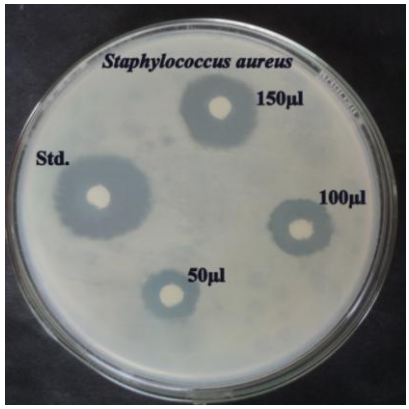
Values were expressed as Mean ± SD.
Bacterial standard - Chloromphenical
Fungal standard - Fluconazole

Figure - : 5 Antimicrobial activities of *Acalypha indica* leaf

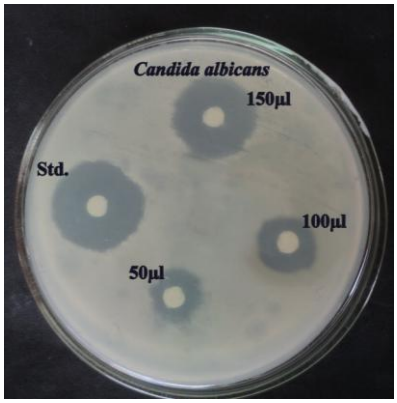
Escherichia coli



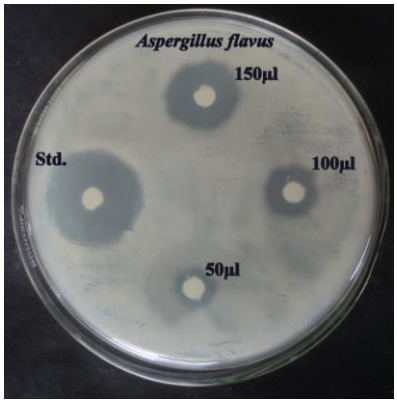
Staphylococcus aureus



Candida albicans



Aspergillus flavus



DISCUSSION

Phytochemical Analysis

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. “Phyto” is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll’s etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural

defense system against predators and diseases, etc., are examples of the roles of secondary metabolites (Sofowara, 1993).

Plants synthesize an array of chemical compounds that are not involved in their primary metabolism. These 'secondary compounds' instead serve a variety of ecological functions, ultimately to enhance the plants survival during stress. In addition these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures. Medicinal plants are assumes greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments (Liu, 2003).

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Acalypha indica* leaf investigated and summarized in Table-1 and fig- 2 and 3. The phytochemical screening methanol extract of *Acalypha indica* leaf showed that the presence of alkaloids, steroids, saponins, flavonoids, terepenoids, phenolics, triterpenoids, carbohydrate, glycosides and anthroquinone while tannin, phlobatannins and protein were absent. Aqueous extract of *Acalypha indica* leaf showed that the presence of protein, steroids, saponins, triterpenoids, phenolics, carbohydrate, anthriquinone, glycosides, flavonoids, terepenoids, while tannin, phlobatannins and alkaloids were absent. Significant amount of Flavonoids (50mg/gm), terpenoids 10(mg/gm) and phenol (110mg/gm) and saponnin (30mg/gm).

Falodun *et al*. (2006) reported the occurrence of flavonoids, saponins, diterpenes and phorbol estersin in the aqueous and methanol extracts of *Euphorbia heterophylla*. Raghavendra *et al*. (2006) examined the powdered leaf material of different solvent of *Oxalis corniculata* and reported the presence of phenols, glycosides, carbohydrates, phytosterols and tannins. Awoyinka *et al*. (2007) extracted eight bioactive compounds from dry leaf of *Cnidoscolus aconitifolius* using water and ethanol. Different extracts of *Semecarpus anacardium* were analysed by Mohanta *et al*. (2007) for its phytochemical properties.

Histochemical Studies

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues (Krishnamurthy, 1998). Histochemical techniques have been employed to characterize structure and development and to study time course of deposition and distribution of major storage compounds such as proteins, lipids, starch, phytin and minerals like calcium, potassium and iron (Krishnan *et al*., 2001). The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other markers. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice. Table 2 and figure 4 represents histochemical studies of *Acalypha indica* leaf powder. This study further confirmed the presence of phytochemicals in *Acalypha indica* leaf extract.

Qualitative Analysis of Vitamins

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The word organic means that the molecules of the substance contain the element carbon. The term also means that vitamins can be destroyed and become unable to perform their functions in our bodies. Too much heat, certain kinds of light and even oxygen can destroy some vitamins. The amounts of vitamins ingested from food are measured in micrograms or milligrams (Okwu, 2004). Vitamin E remains the most mysterious of vitamins. The body needs it but its lack does not lead to any known disease. Vitamin E is the most exploited vitamin in that it is sold as a cure-all and even as an anti-aging potion. Vitamin E, vitamin C and beta carotene are antioxidants. Some studies suggest that the trio might help to strengthen the body's immune system and play a role in cancer prevention (Okwu, 2004). Vitamin C or ascorbic acid, is one vitamin humans cannot make; they have to get it from food. Vitamin C helps hold the cells together, heal wounds and build bones and teeth. The best sources for vitamin C are citrus fruits, strawberries, melons and leafy green vegetables. Vitamin C also helps to absorb and use Iron. It is important to protect the vitamins in fruits and vegetables from being destroyed; simple ways of doing this include refrigeration, washing them before cutting them, storing them in airtight containers and avoiding high temperatures and long cooking times (Okwu, 2003). In the present study vitamin C and E were present and represent in table 3 and fig 5.

Antimicrobial Activity of *Acalypha indica*

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. Emergence of pathogenic microorganisms

that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al*., 2003).

This situation forced scientists to search for new antimicrobial substances with plant origin. Plant extract of *Acalypha indica* was screened against *Escherichia coli* and *Staphylococcus aureus* species of bacteria and *Candida albicans* and *Aspergillus flavus* species of fungi were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Acalypha indica* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 6. The inhibitory activities in culture media of the *Acalypha indica* reported in Table 4 were comparable with standard antimicrobiotic viz. chloromphenical and fluconazole.

CONCLUSION

Determination of the natural phytochemicals and antimicrobial compounds will help to develop new drug candidates for antimicrobial therapy. The most important bioactive compounds are flavonoids, tannins and phenolic compounds present in *Acalypha indica* leaves. These are the important raw materials for drug production with antimicrobial properties for protection against aggressor agents, especially microorganism. Overall, the *Acalypha indica* leaves are a rich source of phytochemicals and potential antimicrobial activity that can be important in infectious disease prevention and health preservation.

REFERENCES

Ammara, H.,Salma R., Farah, D. & Shahid, M.,(2009). Antimicrobial activity of some plant extracts having hepato-protective effects. Journal of Medicinal Plant Research., 3(1): 020-023.

Awoyinka O, Balogun IO and Ogunnowo AA. (2007). Phytochemical screening and *invitro*

Balick JM and Cox PA.,(1996). Plants, People and Culture: the Science of Ethnobotany. New York: Scientific American Library.

Bohm BA, Kocipai-Abyazan R (1994). Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium. Pacific Sci. 48: 458-463.

Falodun, A., Okunroba, L.O. and Uzoamaka, N. 2006.Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of Euphorbia heterophylla L. (Euphorbiaceae). Afr. J. Biotechnol, 5: 529-531.

Ferguson N.M., (1956). A Text Book of Pharmacognosy.New Delhi: Mac Milan Company., 191.

Griggs and Govendarajan., (2001). Turmeric Chemistry, Technology and Quality. CRC Food Sci. Nutr., 12: 199 -301.

Harborne JB (1984). Methods in plant biochemistry, In: Plant phenolics: Academic Press, London, UK.

Hill AF.,(1989). Economic Botany: A Text Book of Useful Plants and Plant Products, second ed. New York: McGraw Hill Book Company, Inc., 560 -562.

Karaman L, Sahin F, Gulluce M, Ogutcu H, Sngul M, Adiguzel A. (2003) Antimicrobial activity of aqueous and methanol extracts of Juniperus oxycedrus L. J Ethnopharmacol.; 85: 231-235.

Krishnamurthy KV. (1998) Methods in plant histochemistry, S. Viswanathan, Chennai. Krishnan S, Ebenezer GAI and Dayanandan P. (2001) Histochemical localization of storage components in Caryopsis of rice (Oryza sativa L.) Curr Sci, 80: 567-571.

Liu C.Z., Murch S.J., El-Demerdash M. (2003) Regeneration of Egyptian medicinal pl ant *Artemisia judaica*L . Plant Cell Rep. 21(6): 525-530

Mann A, Banso A and Clifford LC, 2008. An antifungal property of crude plant extracts from Anogeissus leiocarpus and Terminalia avicennioides. Tanz. J. Health Res., 10 (1): 34-38.

NCCLS. (1993) National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility tests. *PA: NCCLS Publications* 2-5.

Okwu DE. (2003) Investigation into the medicinal and nutritional potential of garcinia Kala (Britter kala) Heckel and Demettia tripetala G.Beaker Ph.D. Dissertation of Michael

Okpara University of Agriculture Umudlike , Nigeria. 5-10.

Patel KK. (2005) Master dissertation. Shorea robustra for burn wound healing and antioxidant activity. Department of Pharmacology, KLESS College of Pharmacy, Karnataka, India, p.33.

Pearson D., The Chemical Analysis of Food, 17th ed. Churchill Livingstone, London. 1976. pp 3-4.

Salie and Simon, Banso n A.,(1996). Inhibitory effect of curcuminoids on MCF 7 cell proliferation and structure-activity relationship.,129: 111- 116.

Sofowara A (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 289.

Sofowara A.,(1982). Medicinal Plants and Traditional Medicinal in Africa. New York: John Wiley and Sons; 256.

Tapsell L.C., Hemphill I., Cobiac L., (2006). Health benefits of herbs and spices: the past, the present, the future. MedicalJournal of Australia. 185(4): 4-24.

Trease GE and Evans WC. (1989) Phenols and Phenolic glycosides. In:Textbook of Pharmacognosy. (12th ed.). Balliese, Tindall and Co Publishers, London pp. 343-383.

Van Wyk B, Gericke N and Van Oudtshoorn B, 2000. Medicinal Plants of South Africa. Briza Publications. Pretoria, South Africa, pp 304- 312.

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