e- ISSN: 2394-5532
p- ISSN: 2394-823X
Scientific Journal Impact Factor: 3.762
International Journal of Applied And Pure Science and Agriculture
www.ijapsa.com

Prevention of Mastitis in Dairy cattle's at Wayand District, Kerala, South India using "Herbalism"

M.D.Dinesh[1*], Ayona George[1], Athira Vijayan[1], C.C.Divya[1], Jyothi Sathyan[1], K.M.Susmitha[1], K.P Sruthi[1], P.S Athira[1] and S. Meenatchisundaram[2]

[1]*Department of Microbiology, Pazhassiraja College, Pulpally, Wayanad- 673 579, Kerala, India*

[2]*Department of Microbiology, Nehru Arts and Science College, Thirumalayampalayam, Coimbatore – 641 105,Tamil Nadu, India*

*corresponding Author

Abstract

Based on oral literature collectively from dairy farmers in Wayanad Disrict, medicinal plants collected from in and around Wayand region. Milk samples are collected from infected cows and the organisms were isolated and identified as Escherichia coli, Klebsiella and Staphylococcus aureus. Inhibitory properties of aqueous extracts of 7 different plant species were tested against Escherichia coli, Klebsiella and Staphylococcus aureus. The determination of the antibacterial activity by agar well diffusion method and 4 plants extracts tested exhibited antibacterial activity against all the isolates. Inhibitory properties of combined aqueous extracts also tested against isolates and showed antibacterial activity against the isolates. On Phytochemical screening indicated the presence of some bacteriocidal compounds which inhibit the growth of isolates from mastitis milk samples.

Key words: mastitis, aqueous extracts, phytochemical screening.

I. Introduction

Dairy farming from being usual family run businesses nowadays has grown tremendously. Dairying is a vital source of additional income to small/marginal farmers and agricultural labourers. In adding together to milk, the manure from animals provides a good resource of organic matter for getting better soil fertility and crop production. Since agriculture is mainly seasonal, there is an opportunity of creating employment all over the year for many persons all the way through dairy farming. Thus, dairy also offer employment throughout the year. The main beneficiaries of dairy programmes are small/marginal farmers and landless labourers. India is gifted with the prime livestock population in the world. Knowledge about the common diseases in dairy cattle's to farmers and their veterinarians help to plan management and prevention strategies that are likely to get better the profitability of the dairy.

Mastitis has been and continues to be recognized as one of the major constraints in the growth of dairy industry in India and abroad. It is also one of the most costly diseases confronting the dairy farmer. Mastitis, the most important deadly disease of dairy animals is responsible for heavy economic losses due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%) (Bhikane and Kawitkar, 2000). Worldwide mastitis is associated with economic losses of $35 billion annually. In India annual economic loss incurred by dairy industry on account of udder infections is estimated to be Rs.6053.21 crores (Dua, 2001).

Mastitis is a complex disease and thus there is no simple solution for its control. More than 250 different microorganisms can cause mastitis. Even no single vaccine is successful to control mastitis due to its multi-etiological nature. Antibiotics ranging from narrow to broad spectrum have been used extensively over the past 40 years in the control of bovine mastitis (Barkema *et al*., 2006). But the problem in dairy animals remained as it was

prior to antibiotic era. The antibiotic treatment may help in minimizing the losses. The prolonged use of antibiotics in the treatment of mastitis has led to an additional problem of the emergence of antibiotic resistant strains, hence the constant concern about the resistant strains entering the food chain (Collins *et al.,* 2010). Till date broad spectrum antibiotics are injected to reduce financial loss. It leads to serious side effects. Therefore alternative therapies are required for the effective treatment of mastitis. The use of vaccination to control infectious diseases in dairy cattle is common and vaccination against mastitis causing pathogens is a control strategy used by some dairy farmers in developed countries. At present in India, no vaccine is available against any of the major mastitis causing organisms although, some efforts were made in past.

The history of "herbalism" is closely tied with the history of medicine from prehistoric times up until the growth of the germ theory of disease in the 19th century. Plants have been used for medical treatments during the human history, and such traditional medicine is still widely used today. The World Health Organisation (WHO) estimates that 80% of the people living in developing countries almost exclusively use traditional medicines. This means approximately 3300 million people use medicinal plants on a regular basis. Medicinal plants used in traditional medicine should therefore be studied for safety and efficacy. Hence in our present study we investigate study on mastitis cases in Wayand and the antibacterial activity of the aqueous extracts of selected medicinal plants against mastitis causing pathogens.

II. Materials and methods

Collection of medicinal plants: Wayanad district of Kerala, South India, is famous for their traditional way of treatment based on natural herbs. Traditional farmers in Wayand formulate there therapeutics using indivugal ingredients combine with medicinal herb for the conditions of sickness. Based on oral literature together from dairy farmers in Wayanad Disrict, medicinal plants obtained from in and around Wayand region were used for our study. (Table- 1)

Table 1- Medicinal plants used

S.l no	Common name	Scientific name	Parts used
1	Aloe vera	*Aloe Barbadensis Miller*	Leaf
2	Aatha	*Annona squamosa*	Leaf
3	Aruvep	*Azadirachta indica*	Leaf
4	Niruri	*Phyllanthus niruri*	Leaf
5	Kuruvillakadukka	*Terminalia Chebula*	Seed
6	Muthira	*Macrotyloma uniflorum*	Seed
7	Manjal	*Curcuma longa*	Seed

Isolation and Identification of Mastitis causing organisms

Mastitis infecting bacterial strains was isolated from milk samples from the infected cows. Milk samples were collected from various veterinary dispensaries in and around Pulpally, Wayand, Kerala. Following collect the milk sample isolation and identification of bacteria was completed on the root of morphological, biochemical and cultural characteristics. (Table - 2)

Preparation of Crude Plant Extract: Medicinal plants obtained from in and around Wayand region were prepared by the method of Uhegbu *et al*., (2005) using distilled water as the solvent. 20 g of powdered sample of the herb was extracted by soaking in 180 mL of distilled water in a beaker, stirred for about 6 min and left overnight. Thereafter, the solution

was filtered using filter paper (Whatman No. 1) and the extracts were evaporated to dryness under reduced pressure below 40°C.

Antimicrobial activity of various plant extracts against clinical pathogens

Well diffusion method

The agar well diffusion method was adopted according to Kavanagh, (1972) to assess the antibacterial activity of the prepared extracts. Loop full of bacterial stock suspensions was thoroughly mixed with 100 ml of sterile nutrient agar and kept for overnight incubation. 20 ml of the Muller Hinton agar were distributed into sterile Petri dishes. The agar was left to set. Bacterial suspension was adjusted according to Mac-Farland standard 0.5 and swab culture was made on the respective plates. In each of these plates 4 wells were made by using a sterilized 6 mm cork borer and the agar discs were separated. The wells were filled different extracts of 20µl, 40µl, 60µl, 80µl respectively and allow diffusing of plant extract into the medium for about 45minutes. The plates were followed by incubated at 37 C for 24 h for bacteria after which microbial growth was determined by measuring the diameter of the inhibition zone (mm) using a translucent scale. Each extract was analyzed in triplicate, the mean values are presented.

Phytochemical screening: The crude plant extract was subjected to phytochemical analysis for detecting the chemical compounds in it.
Test for Saponins - About 0.5gm of each plant extract was shaken with water in a test tube. Frothing, which persist on warming was taken as preliminary evidence for the presence of saponins (Kokate., 1999).

Test for Tannins - About 0.5gm of plant extract was stirred with 1ml of distilled water, filtered and a few drops of 1% ferric chloride was added to the filtrate. A blue-black, green or blue-green precipitate was taken as the evidence for the presence of tannins (Mace., 1963).

Test for Anthraquinone - About 0.5gm of extract was taken and 5ml of chloroform was added and shaken for 5min. The extract was filtered and filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in ammoniacal layer indicates the presence of anthraquinone.

Test for Flavanoids - About 0.5gm of plant extracts were treated with 2ml of 2% sodium hydroxide solution. An intense yellow colour which turned to colourless on the drop wise addition of dilute acid indicates the presence of flavanoids. (Trease and Evans, 2002).

Test for Phenol - To the plant extracts dissolved in water equal amount of ferric chloride was added. Deep bluish green colour indicates the presence of phenol.

Salkowsky test - 0.5gm of plant extract was dissolved in 2ml of chloroform. 2ml of conc. Sulphuric acid was carefully added to form a lower layer (chloroform layer). A reddish-brown colour at the interface indicates the presence of a steroidal ring. (Sofowora, 1993).

Test for Proteins - Millon's test: To 2ml of plant extract, added 2ml of Millon's reagent and observed for two minutes for the formation of white precipitate. On gentle heating which may turned to red indicates the presence of proteins in it (Rasch and Swift., 1960).

Test for Amino acids - Ninhydrin test: To 2ml of plant extract, added 2ml of Ninhydrin reagent. Violet colour indicates the presence of amino acid / proteins in it.

Test for Sugars - About 0.5 ml of the extract dissolved in water was taken. The volume was made upto 1 ml with distilled water. 4 ml of the Anthrone reagent was added. It was heated for 10 minutes in boiling water bath with lids closed. The tube was cooled rapidly. Blue black colour indicates the presence of sugars (Yemm and Wills., 1954).

Test for Reducing Sugars - To the 5ml of Benedict's reagent, added 2ml of aqueous plant extract and boiled for 5min in boiling water bath. Red precipitate indicates the presence of reducing sugar.

III. Results and Discussion

Mastitis continues to be the most frequent and costly disease of dairy cattle. Financial losses due to mastitis occur for both subclinical and clinical stages of the disease. Losses caused by subclinical mastitis are well documented. (Hortet and Seegers, 1998). Losses caused by clinical mastitis include discarded milk, transient reductions in milk yield and premature culling (Fetrow, 2000).

In India, *Staphylococcus*, *Streptococcus* and *E.coli* generally cause 90-95% of all infections of mammary gland (mastitis). The goal of every dairy farmer should be to minimize the number of organisms permitted to come into contact with the teats. In India, the teat dipping as a preventive measure is not regularly practiced by dairy farmers; hence, it is essential to educate the farmers regarding the risk factors of mastitis and also about teat dipping (Kavitha *et al.*, 2009). Estimates show that on the standard a district affected with mastitis results in a 30% reduction in efficiency and an affect cow is estimated to loss 15% of its production (Blood & Radostits ,1989). According to the survey reports from District Veterinary and dishusbandry Department, Kalpatta, Wayanad number of mastitis cases is reported in Kalpata in the year of 2015. The mastitis cases reported in Wayand district hospitals from the period of June- 2015 to December – 2015 (Dinesh *et al.,* 2016).

Mastitis treatment with antibiotics leads to the development of antibiotic resistant strains and consumer health problem. Clinical mastitis treatment protocols are based on treating the mastitic quarter to clear the infection and return the milk and quarter to normal. Broad spectrum antibiotics are used widely for the current therapies. The medicinal plants are in great demand in traditional system of medicine i.e. Ayurveda, Siddha and Unani Tibb as well as folklore prescriptions. The modern pharmaceutical industry also requires a large quantity of authentic plants for manufacture of drugs. Use of herbal medicine, sometimes also referred as '**Herbalism**', is an ancient practice for healing. (Dinesh *et al.*, 2016).

In our present research we investigate the antibacterial activity of the aqueous extracts of preferred indigenous medicinal plants against mastitis causing pathogens. Mastitis causing pathogens are isolated from milk samples from mastitis infected cows in and around Wayand region. After collecting the milk sample Isolation and identification of bacteria was finished on the basis of morphological, cultural and biochemical characteristics. We isolated different pathogens in collected milk samples and the isolates were identified as *Escherichia coli, Klebsiella and Staphylococcus aureus.* A total of 7 aqueous extracts from 7 different plant species were investigated. Each plant extracts were tested at different concentrations (20, 40, 60 and 80µl/ml) to see their inhibitory effects against isolated pathogens. The most prominent activity with inhibition zones of more than 11.5 mm was shown by *Phyllanthus niruri* followed by *Terminalia Chebula* (7.8 mm) and *Annona squamosa* (7.8 mm). When the concentration of the extracts was decreased, slight decrease in inhibition zones were observed.

The present experimental results indicate that out of 7 aqueous extracts from 7 different plant species, 4 plant extracts tested exhibited an antimicrobial effect against all the pathogens. These results showed that the extracts from *Annona squamosa*, *Phyllanthus niruri*, *Terminalia Chebula and Macrotyloma uniflorum* possessed antimicrobial activity against all pathogens. The result from this preliminary study indicates that these plant extracts could be used for therapeutic purpose in case of mastitis in dairy cattle's. Further investigations are needed for identification and purification of the specific antimicrobial components from these plants against mastitis causing pathogens.

Table 2 - Morphological characterization of Collected Milk samples

	Samples	Morphology characterisation
1	Sample 1	Gram negative rods
2	Sample 2	Gram negative rods
3	Sample 3	Gram negative rods
4	Sample 4	Gram negative rods
5	Sample 5	------
6	Sample 6	Gram positive cocci
7	Sample 7	Gram negative rods
8	Sample 8	Gram negative rods
9	Sample 9	--
10	Sample 10	---

Table – 3 – Biochemical characterisation

Sample	Indole Test	Methyl red	Voges Proskauer Test	Citrate utilization Test	Urease Test
Sample 1	+	+	-	-	-
Sample 2	-	-	-	+	-
Sample 3	+	+	-	-	-
Sample 4	+	+	-	-	-
Sample 5					
Sample 6	Cultural characterised on Manitol salt agar plates				
Sample 7	-	-	-	+	-
Sample 8	+	+	-	-	-
Sample 9					
Sample 10					

Table: 4 - Antibacterial activity of crude extracts of various medicinal plants against E. coli, *Klebsiella* and *Staphylococcus aureus* - Well cut Method

Plant extracts	Conc. µl/ml	Zone of inhibition (mm)		
		E.coli	*Klebsiella*	*Staphylococcus aureus*
Aloe Barbadensis Miller	80	-	-	-
	60	-	-	-
	40	-	-	-
	20	-	-	-
Annona squamosa	80	7.8	5.8	7.4
	60	5.9	4.3	5.2
	40	3.2	2.8	3.4

	20	2.8	2.0	2.2
Azadirachta indica	80	4.8	-	5.6
	60	3.8	-	4.7
	40	2.3	-	3.6
	20	2.0	-	2.0
Phyllanthus niruri	80	11.5	10.4	11.2
	60	9.8	8.2	10.4
	40	6.9	5.3	7.4
	20	5.2	4.2	6.2
Terminalia Chebula	80	7.1	6.2	7.8
	60	5.2	4.9	6.4
	40	3.5	3.8	4.6
	20	3.2	2.4	3.4
Macrotyloma uniflorum	80	5.7	6.4	7.1
	60	4.4	5.0	4.9
	40	3.4	3.6	3.8
	20	2.0	2.2	2.1
Curcuma longa	80	-	-	-
	60	-	-	-
	40	-	-	-
	20	-	-	-

Figure 1- Comparison of antimicrobial activity of crude extracts of various plants against *E.coli*

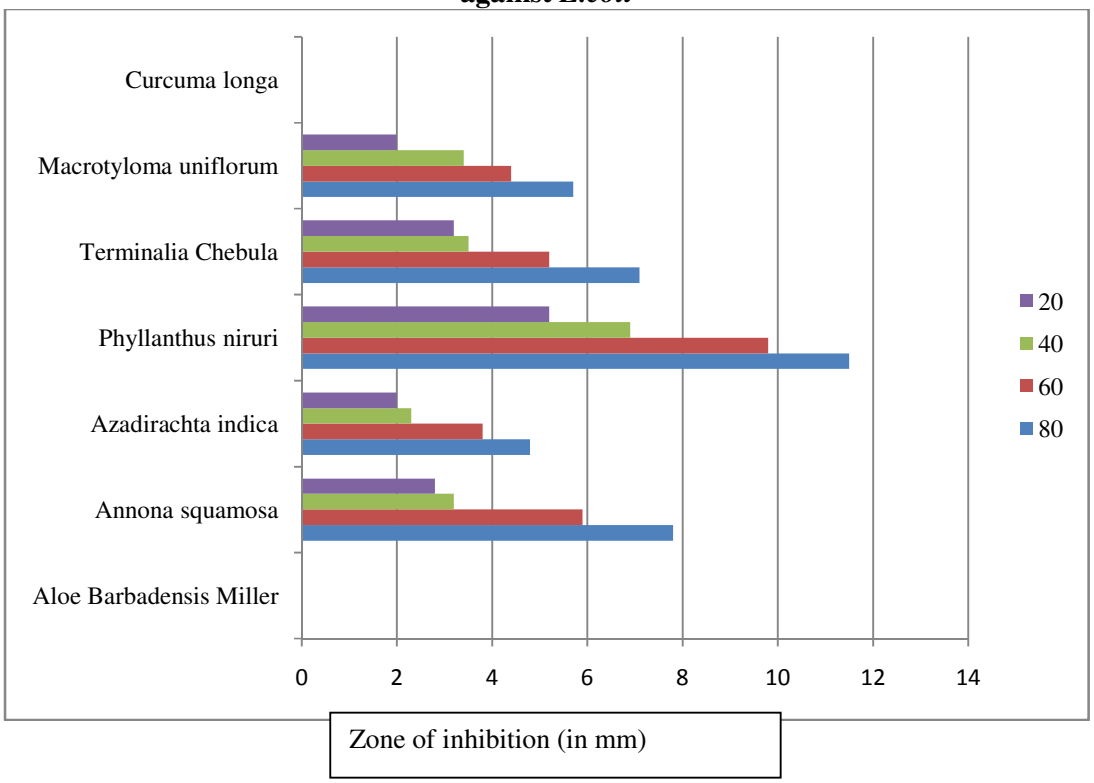

Figure 2- Comparison of antimicrobial activity of crude extracts of various plants against *Klebsiella*

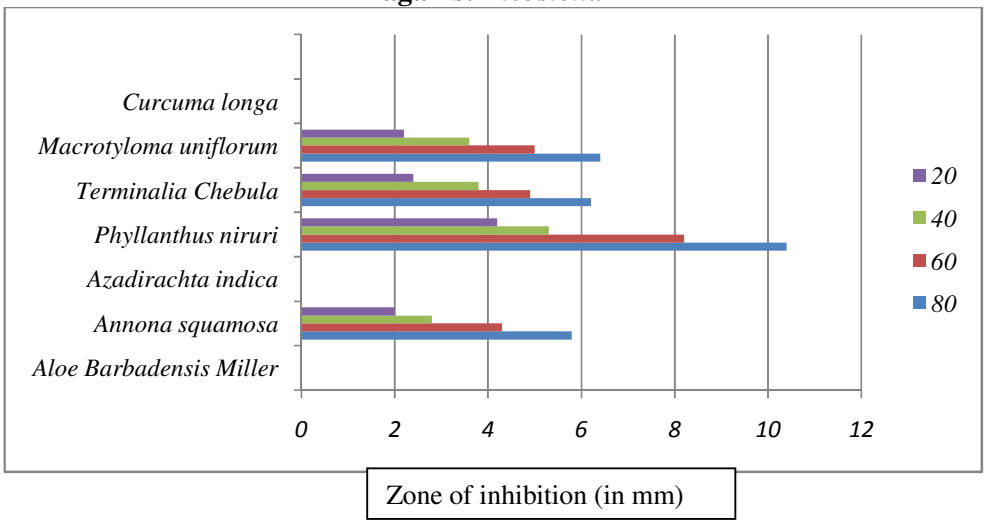

Figure 3- Comparison of antimicrobial activity of crude extracts of various plants against *Staphylococcus aureus*

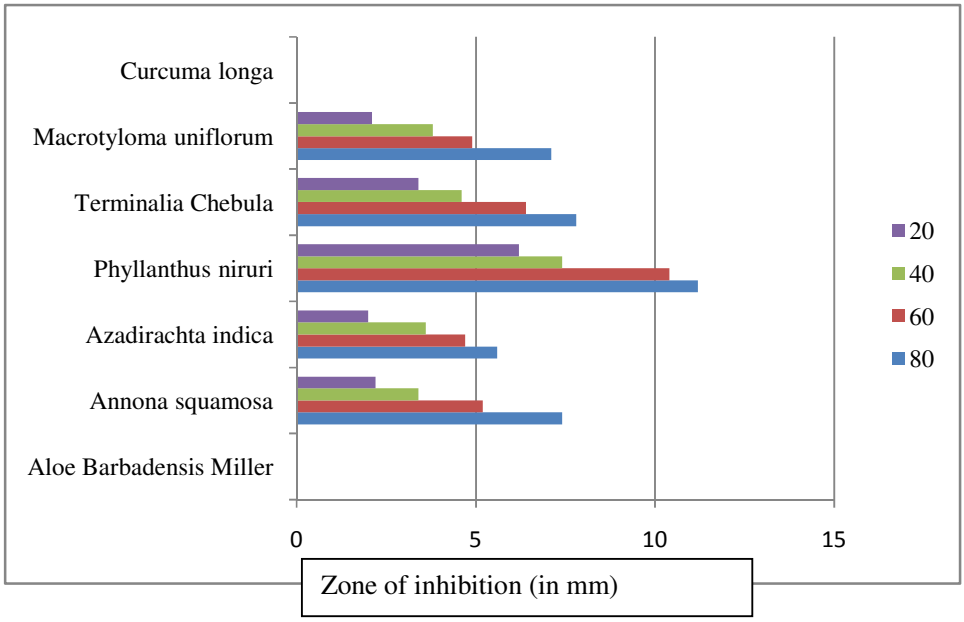

Table – 5 – Phytochemical analysis

Tests	*Aloe Barbadensis Miller*	*Annona squamosa*	*Azadirachta indica*	*Phyllanthus niruri*	*Terminalia Chebula*	*Macrotyloma uniflorum*	*Curcuma longa*
Saponin	+	-	-	-	+	+	-
proteins	-	-	-	-	-	-	+

Tannins	-	+	-	-	+	-	-
Anthraquinone	-	-	-	+	+	-	+
Flavanoids	+	-	-	-	-	+	-
Phenols	-	-	+	+	+	-	-
Salkowsky test	-	+	-	-	+	-	+
Anthrone test	-	+	-	-	-	-	+

Abbreviation: - + (Positive**) - (**Negative**)**

Bibliography

[1] Blood , D. C .and Radostits , O . M .(1989) Veterinary Medicine. Bushnell , R . B . (1980 Am . vet . med . Assoc . , 176 , 746.
[2] Fetrow, J. 2000. Mastitis: an economic consideration. pp 3-47 in Proceedings of the 29th annual meeting of Natl. Mast. Coun., Atlanta, GA, Natl Mast Coun. Madison, WI.
[3] Hortet P., Seegers H., Milk yield loss and related composition changes resulting from clinical mastitis in dairy cows: a review, Prev. Vet. Med. 37 (1998) 1–20.
[4] Kavitha, K.L., K. Rajesh, K. Suresh, K. Satheesh and N. Syamasundar, 2009. Buffalo mastitis- risk factors. Buffalo Bull., 28: 134-137. Make references like this style.
[5] Uhegbu, F.O., I. Elekwa and C. Ukoha, 2005. Comparative Efficacy of crude Aqueous Extract ofMangifera indica, carica papaya and sulphadoxine pyrimethamine on the mice infested with malariaparasite *in vivo.* Global J. Pure Appl. Sci., 11: 399-401.
[6] Barkema, H.W., Y.H. Schukken and R.N. Zadoks. 2006. Invited Review: The role of cow, pathogen and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. *J.Dairy.Sci*. **89**: 1877-1895.
[7] Bhikane, A.V. and S.B. Kawitkar. 2000. Hand book for Veterinary Clincian. Venkatesh Books. Udgir, India.
[8] Dua, K. 2001. Incidence, etiology and estimated economic losses due to mastitis in Punjab and in India-An update. *Indian Dairyman*. **53(10)**: 41-48.
[9] Dinesh. M.D, Ayona George, Athira Vijayan, C.C. Divya, Jyothi Sathyan, K.M. Susmitha, Smitha Chacko, M.C. Vijisha and S. Meenatchisundaram. 2016. Study on Mastitis in Dairy Cows at Wayanad District, Kerala, South India. *Int. J. Curr. Res. Biosci. Plant Biol.* 2016, 3(2): 65-68.
[10] Collins, N., Ateba, Moses Mbewe, Modisane, S., Moneoang, Cornelius, C. and Bezuidenhout (2010). Antibiotic resistant S. aureus isolated from milk in the Mafikeng area, North West province, South Africa. S. Afr. J. Sci., 106 : 11 - 12.
[11] Kavanagh F (1972). Analytical Microbiology, vol. 2, Academic Press, New York.
[12] Kokate C.K., Purohit A.P., Gokhale S.B. Pharmacognosy 2009: P-11.53.
[13] .Mace ME (1963) Histochemical localization of phenols in healthy and diseased tomato roots. Phytochem. 16, 915-925.
[14] Trease GE, Evans WC. Pharmacognosy. 15th Ed. London: Saunders Publishers; 2002. pp. 42–44. 221–229, 246–249, 304–306, 331–332, 391–393.
[15] Sofowora, A. (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd (Pub.), Ibadan.
[16] Rasch E, Swift H (1960). Microphotometric analysis of the cytochemical Millon action. J. Histochem. Cytochem., 8: 4-17.
[17] Yemm, E.W. & Willis, J. (1954). The estimation of carbohydrates in plant extracts by anthrone. Journal of Biochemical, Vol.57, No.3, p. 508-514, ISSN 0264-6021.

