International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 1104-1110
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Phytochemical Analysis and Anti-bacterial activity in Stem Bark of *Terminalia tomentosa* Wight and Arn.

V. Asha Krishna* and P. Sujathamma
Department of Biosciences and Sericulture, Sri Padmavati Mahila Visvavidyalayam, (Women's University), Tirupati - 517 502, Andhra Pradesh, India.

Received: 24 Mar 2019 / Accepted: 26 Apr 2019 / Published online: 1 Jul 2019
***Corresponding Author Email:** ashakrishnanine@gmail.com

Abstract
Phytochemicals are the chemical compounds of the plants which possess the medicinal role. *Terminalia tomentosa* tree is known to be a medicinal plant mainly its bark which is used since time immemorial. The aim of the study was intended to explore various phytoconstituents and bactericidal potential in stem bark of *T.tomentosa.* Different fractions of stem bark revealed the presence of bioactive constituents such as alkaloids, flavonoids, phenols, steroids, saponins, tannins, anthocyanins and lignins whereas indices and glycosides were barred in all the extracts. Anti-bacterial activity of aqueous, ethanol and methanol extracts of stem bark of *T.tomentosa* samples were studied with respect to pathogenic bacteria *Staphylococcus aureus, Escherichia coli and Klebsella pneumoniae.* Ethanolic fraction exhibited highest zone of inhibition on *S.aureus* and lowest was observed against in aqueous against *K.pneumoniae.* These results are suggestive for the global thrust areas for drugs as well as in industrial application.

Keywords
Anti-bacterial activity, Medicinal values, Secondary metabolites, Stem bark, *Terminalia tomentosa*.

*****

INTRODUCTION
Nature is providing lavish of plant wealth which constituents enormous medicinal values. Plants are potentially useful chemical compound reservoirs which serve as drugs for powerful treatment of various ailments [11]. Phytochemicals are the chemical compounds of the plants which possess the medicinal role. The history of medicine includes many ludicrous therapies. In India since ancient times, drugs of herbal origin have been used in traditional systems of Unani, Ayurveda and Siddha. Medicinal plants sector has traditionally occupied an important position in the socio-cultural, spiritual and medicinal area of rural and tribal lines of India. The global thrust areas for drugs from medicinal plants include disease conditions, whose incidence is unavailable or unsatisfactory.

An extensive literature survey of *T.tomentosa* revealed a variety of chemical constituents. The drug of natural and allopathic medicinal value is due to the presence of various active substances being as alkaloids, flavonoids, steroids, glycosides, phenolic compounds, resins, phytosterols, volatile oils, gums, tannins, saponnins and lignins etc.

T.tomentosa trees are found in various regions of the country where they are lumbered and well recognized in tropical tasar silk industry as the most important primary food plant. It has been regarded as one of the India's prime commercial woods and is protected. *T.tomentosa* is casually called as 'crocodile bark tree' due to its characteristic bark pattern. *T.tomentosa* is native to Southern and Southeast Asia in India, Nepal, Bangladesh, Myanmar, Thailand, Laos, Combodia and Vietnam [21].

Terminalia tomentosa tree is known to be a medicinal plant mainly due to its bark. The bark and fruits of some species of the genus are used medicinally since time immemorial particularly due to their cardiotonic and diuretic properties. *T.tomentosa* bark decoction has been mentioned in *Charaka Samhita* for treatment of rheumatism, fever, urinary diseases and diabetes. It is used for in atonic diarrhea and generally for indolent ulcers. Bark powder is an excellent remedy in vertigo, piles, constipation and chronic dysentery. It has great importance in Ayurveda to treat bone fractures, haemorrahages and bronchitis. In Siddha form of medical treatment, the bark, gum and leaves of asan is used to treat fever and ear ache. Bark is useful in vitiated conditions of pitta, cardiopathy, strangury, haemoptysis, dysentery, cough, bronchitis, verminosis, leucorrhoea, gonorrhea, liver troubles, blood diseases and white leprosy etc. Bark of *T.tomentosa* is used in Indian traditional and folklore medicine for wound healing, GI disorders and anti-inflammatory purposes but it lacks scientific evidences [13].

As per literature reviewed Joshi *et al*., [9] stated that plant of *T.tomentosa* is known to possess many pharmacological properties like antifungal, antioxidant, anti-hyperglycemic, anti-diarrhoeal and antileucorrheal.

The present work is aimed to determine the phytochemical components and anti-bactericidal potential in stem bark of *T.tomentosa* as indices of producing plant secondary metabolites which have medicinal values as well as application in industries.

MATERIALS AND METHODS

Sample collection and Preparation of bark extract

Bark of *T.tomentosa* was collected from Seshachalam forests of Eastern ghats of TIRUMALA HILLS, Chittoor district. Bark was shade dried and pulverized to a coarse powder and sequentially extracted with aqueous, methanol, alcohol, ethyl acetate, chloroform, petroleum ether, benzene and hexane by using standard procedures to identify the constituents. For extraction 5 gms of bark powder was taken into 250 ml of each different solvents and were soaked for 48hrs. Finally, these crude extracts were filtered and used for preliminary screening.

Phytochemical analysis was conducted for the identification of bioactive constituents in multihued extracts of the bark sample as per Harborne [8] standard procedure.

Determination of Anti - bacterial assay

Anti-bacterial activity conducted with aqueous, methanol and ethanol extracts of stem bark of *T.tomentosa*. Clinical strains of *Staphylococcus aureus, Escherichia coli* and *Klebsella pneumonia,* were two gram-positive and one gram-negative bacteria obtained from Department of Microbiology, Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh. Streptomycin, Penicillin and Ciprofloxacin were the standard drugs as positive controls used for this assay. Bacterial cultures were collected in nutrient broth agar medium by agar well diffusion method. These wells were repleted with sample extract and control of about 100 µl. The plates were incubated at 37^0 C for 24 h and zone of inhibition was exhibited as diameter (mm) as they were produced around each well by the plant extracts and antibiotics.

RESULTS

Phytochemicals in stem bark

The concentration of yield extraction differed among the solvents depending on their polarity. phytochemical screening of the crude extracts of bark of *T.tomentosa* revealed the presence of different kinds of chemical constituents. The existence of bioactive compounds indicate the medicinal value of various extracts of *T.tomentosa*, because their possible use as natural additives emerging from a burgeoning tendency to take over from synthetic antioxidants and antimicrobials with natural ones. The presence or absence of phytochemicals indicated as high, moderate and absent. These results were shown in Table 1.

Table:1 Phytochemical analysis in different extracts of stem bark of *Terminalia tomentosa* Wight and Arn

Fractions	Aqueous	Methanol	Alcohol	Ethyl acetate	Chloroform	Petroleum ether	Benzene	n-Hexane
Alkaloids	++	+	++	++	++	-	++	-
Flavanoids	++	++	++	-	-	-	-	-
Phenols	++	++	++	-	-	-	-	
Steroids	+	++	++	-	-	-	-	-
Saponins	++	-	-	-	-	-	-	-
Tannins	++	++	++	-	-	-	-	-
Antho cyanidines	++	+	++	-	-	-	-	-
Lignins	++	++	++	-	-	-	-	-
Indoles	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-

High = ++; Moderate = +; absent = -

Aqueous and methanol extract of bark of *T.tomentosa* contain alkaloids, flavonoids, phenols, steroids, saponinns, tannins, anthocyanin's and lignin's. Alcohol and methanol extract also possess all these phytoconstituents except saponins. Ethyl acetate, chloroform and benzene resulted presence of alkaloids but absent in petroleum ether and n-hexane. Aqueous gives positive results for all the tests but barred for indoles and glycosides.

Alkaloids array high concentration in all extracts except in petroleum ether and n-hexane. Flavonoids, phenols, tannins, anthocyanin's and lignin's were present in aqueous, methanol and ethanol fractions which exhibited high concentration. Steroids were too highly concentrated in methanol and ethanol but moderate in aqueous. Saponins were observed only in aqueous which possessed high concentration whereas indoles and glycosides were not present in all the extracts.

Anti-bacterial susceptibility against *T.tomentosa* stem bark

The results of the anti-bacterial activity of the Aqueous, Methanol and Ethanol fractions at a concentration of 100µl/well against all bacteria have been found. The zone of inhibition was measured in mm and results were shown in Table 2.

Table: 2 Anti-bacterial activity of Aqueous, Methanol and Ethanol fractions against tested microorganisms

Sno	Microorganism	Zone of inhibition (mm)					
		Extract fractions			Control		
		Aqueous	Methanol	Ethanol	Streptomycin	Penicillin	Ciprofloxacin
1	*Staphylococcus aureus*	13.93 ± 0.66	19.1 ± 0.20	23.46 ± 0.53	22.3 ± 0.18	16.33 ± 0.33	31. 33 ± 0.35
2	*Esherleshia coli*	13.06 ± 0.17	14.03 ± 0.14	21.86 ± 0.13	21.8 ± 0.11	15.93 ± 0.06	30.83 ± 0.44
3	*Klebsella pneumoniae*	5.23 ± 0.14	13.06 ± 0.06	14.13 ± 0.24	16.33±0.33	13.26 ± 0.37	19 ± 0.11

All values are represented as mean ± SD (n = 3)

The three (Aqueous, Methanol and Ethanol) stem bark extracts have inhibited the growth of both gram -positive and gram-negative bacteria such as *staphylococcus aureus, Escherichia coli* and *Klebsella pneumoniae*. Aqueous fraction (5-13 mm) showed mild anti-bacterial activity whereas ethanol (14-23 mm) exhibited higher than methanol (13-19 mm) which shows moderately. In case of antibiotics, streptomycin (16 -22 mm) exhibited moderately, this was followed by penicillin (13-16 mm), whereas ciprofloxacin (19-31) possessed highest zone. Ciprofloxacin exhibited highest zone of inhibition compared with antibiotics and also as well as with the extracts.

Among the three extracts, ethanol exhibited highest zone of inhibition on *S.aureus* (23.46) followed by *E.coli* (21.86). In case of methanol also, *S.aureus* (19.1) showed highest inhibition zone followed by *E.coli* (14.03). With the antibiotics Ciprofloxacin possessed highest zone of inhibition on *S.aureus* (31.33) followed by *E.coli* (30.83). Secondly, streptomycin again on *S.aureus* (22.3) followed by *E.coli* (21.8).

It was found that aqueous, methanol and ethanol extracts of *T. tomentosa* and antibiotics of streptomycin, penicillin and Ciprofloxacin exhibited anti-bacterial activity. In all the above, ciprofloxacin possessed highest zone of inhibition against *S.aureus* (31.33) followed by *E.coli* (30.83). The inhibition was

minimum in aqueous against *K.pneumonia* (5.23) followed by *K.pneumonia* (13.06) in methanol and *E.coli* (13.06) in aqueous.

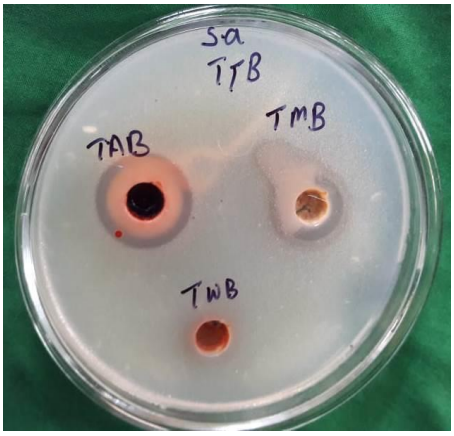

Fig: 1 Anti-bacterial activity of Aqueous, Methanol and Ethanol fraction against *S.aureus*

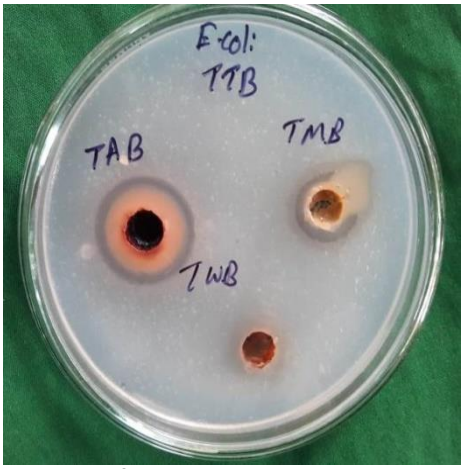

Fig: 2 Anti-bacterial activity of Aqueous, Methanol and Ethanol fraction against *E.coli*

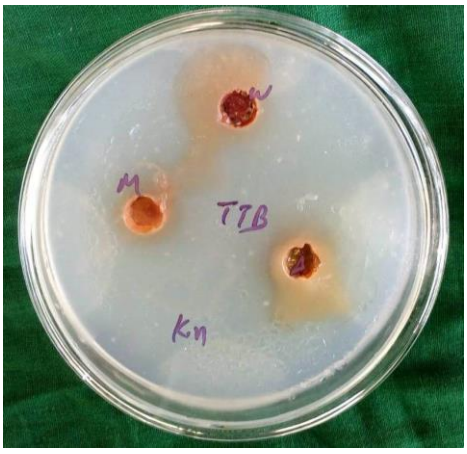

Fig: 3 Anti-bacterial activity of Aqueous, Methanol and Ethanol fraction against *K.pneumoniae*

DISCUSSION

From all the different extracts of bark powder of *T.tomentosa* indicated the presence of phytoconstituents like alkaloids, flavonoids, phenols, steroids, saponins, tannins, anthocyanin's and lignin's. Similar results were observed by Shivaji [20]

who has carried out preliminary phytochemical screening by methanolic extract on stem bark of *T.tomentosa*. Joshi *et al.,* [9] have also observed the same results who have undertaken phytochemical investigation of ethanolic extract of the stem bark of *T. tomentosa* Rox (ex DC) Wight & Arn. The function of alkaloids in plants are still widely vague, although individual substances have been reported to be involved as growth regulators or as insect repellents or attractants. Alkaloids detection is especially important in forensic medicine, a particularly being described by Clarcke [6]. VIsweswari *et al.,* [27] stated that many alkaloids are anaesthetics which have calming effects on psychotic or hypertensive patients and can also be used to treat psychiatric and palpitation.

In plants flavonoids are most common which possess ample of biochemical and pharmacological effects including anti-oxidation, anti-inflammation, antiplatelet and anti-allergic effects. Asif and Khodadadi [2] stated that compounds of flavonoid plays a vital role as a health protecting factor and reduce the risk for various chronic disorders including cancer and cardiac diseases.

Plant phenols can be a considerable nuisance, the term phenolic compound embraces a wide range of plant substances. When plant cell constituents come together and the membranes are destroyed during isolation procedures, the phenols rapidly complex with proteins and as a result, there is often inhibition of enzyme activity in crude plant extracts. Phenols are antiseptic and reduce inflammation when taken internally. Polyphenols act as antioxidants, which protect cells and body chemicals against damage, caused by free radicles that contribute to tissue damage in the body. Rahaman and Visweswari *et al.,* [15, 27] reported that these compounds deactivate the substances that promote the growth of tumors.

The anthocyanin's are the most important and widespread group of colouring matters in plants. The anthocyanin's are all chemically based on a single aromatic structure, that of cyaniding. There are six common anthicyanidins. Sharad and Tanya [18] stated that anthocyanin's have been reported to be an antidiabetic and insulinotropic agents.

Saponins have antimicrobial and especially antifungal activities as one of the characteristic properties [24, 26]. The crude drugs which contain saponins are generally used for their detergent properties, and some of them which give less irritating effects on oral administration [19] are employed as expectorant and antitussive agents.

There have been many reports on the enthno-medicinal usage of the *T.tomentosa* and traditionally plant parts such as bark, leaves, roots, etc., have been used to treat various disordres.

Asquish and Butler [3] have reported that the tannins play major role in the treatment of infectious diseases and have shown antioxidant and protein precipitating properties. Pettit *et al.,* [14] and Kandil and Nassar [10] have reported that tannins showed anticancer activity. Chaudhari and Mengi [5] opined that tannins may be responsible for its wound healing, astringent and antimicrobial activity.

Scientists have great interest in the field of research of biologically active natural compounds for new sources of drugs, useful in controlling diseases.

Taking note of the emerging peril of adverse side effects and drug resistant pathogens all over the world, it is high time to look for novel strategies based on traditional plant based products to microbial infections. Globally, India is particularly privileged in disparate traditional health care systems.

Some chemical substances are brought forth by the plants which lies medicinal value of plants, these chemicals called "secondary metabolites". Chemical or synthetic drugs and antibiotics have been used for treatment of various ailments. In general, these compounds effectively inhibit and/or stop microbial nucleic acids, proteins and cell walls. Presence of phenolics and polyphenols in the plants are known to be toxic to the microorganisms [12].

Chemical composition of this tree is triterpene carboxylic acid, tomentosic acid [16, 21]. A new terpene glycoside, 2α, 3β, 19α – trihydroxy – olean – 12-en-28-oic acid methylester 3β-O-rutinoside, a new flavanone, 8-methyl-5, 7, 2', 4' – tetramethoxyl-flavanone and new chalchone glycoside, 2-O-β-glucosyloxy - 4, 6, 2', 4'-tetramethoxy chalchone have been reported [22, 21]. Shivaji *et al.,* [20] reported that heartwood contains lactone, terminolic acid, triterpinoids, β-sitosterol, tomentosic acid, oleanolic acid, maslinic acid and arjunolic acid.

From the ethanolic extract of stem bark of *T.tomentosa,* Joshi *et al*., [9] isolated and characterized chemical entities which include 4-Methyl-4-hydroxymethylne - 6β – (10 – methyl octanyl), cyclohexane (Arjuna homoses quiterpinol), di – n – octyl phthalate, di isobutyl phthalate and butyl phthalate.

Fakruddin *et al* [7] stated that *Terminalia arjuna* would be a good anti-bacterial drug in the treatment of *Vibrio cholera* infections. Meriga *et al* [13] stated that ethanolic fraction of *T.tomentosa* bark may be treated as a potential successor to develop anti-obesity drugs. According to some references, the

stem bark of *Pterocarpus santalinus* inhibits the growth of *S. aureus*. *Vitex doniana* stem bark noticed bacterial potential against *S. aureus* in methanolic extract. Stem bark of *Holarrhena antidysenterica* possess anti-bacterial potential against enteric pathogen *E.coli* [4]. Sangeetha *et al* [17] also recorded the bacterial potential of *Cassia fistula* against the bacteria *K.pneumoniae.* Udaysing and Dattatraya [26] found that *S. aureus* was highly inhibited and showed maximum inhibition zone and *K.pneumoniae* found resistant with minimum zone of inhibition in bark of *T.arjuna.* The aqueous and methanol fractions of *Terminalia arjuna* bark showed significant zone of inhibition against twenty-two bacteria. Gram-positive strain of *Staphylococcus* exhibited a highest inhibition zone and noted that gram-negative bacteria would be more liable to antimicrobials in comparison to the gram-negative bacteria which possess a thick barrier of lipopolysaccharide layer in the cell surface [23]. The obtained results can be used for further identification of bioactive compounds from the extracts and also may possibly be useful for finding the notable drugs having anti-bacterial properties in *T.tomentosa* as agent for the therapy of infectious diseases.

CONCLUSION

The present study carried out to reveal the presence of various phytoconstituents from all the extracts that have medicinal importance since time immemorial. It is important to isolate the active anti-bacterial constituents of *T.tomentosa.* These results are suggestive for the global thrust areas for drugs as well as in industrial application. Though some studies revealed the role of phytoconstituents of *T.tomentosa*, but information available is scarce. There are still enormous and conclusive studies require on efficacy, safety and toxicity of the compounds for better understanding and satisfactory results.

REFERENCES

[1] Anjum G, Anita S, Amey S and Rajesh D., Non - targeted identification of compounds from regenerated bark of *Terminalia tomentosa* by HPLC – (+) ESI – QTOFMS. Journal of Pharmacy research, 6: 415-418, (2013)

[2] Asif M and Khodadadi E., Medicinal uses and chemistry of flavonoid contents of some common edible tropical plants. J. of Paramedical Sciences, 4 (3): 119-138, (2013)

[3] Asquith TN and Butler LG., Interaction of condensed tannins with selected proteins. Phytochemistry, 25 (7): 1591-1593, (1986)

[4] Ballal M, Srujan D, Bhat KK, Shirwalkar A, Shivananda PG., Antimicrobial activity of *Holarrhena antidysenterica* (Kurchi) against enteric pathogens. Indian journal of pharmacology, 33: 392-93, (2001)

[5] Chaudhari M and Mengi S., Evaluation of phytoconstituents of *Terminalia arjuna* for wound healing activity in rats. Phytotherapy Research, 20:799-805, (2006)

[6] Clarke ECG., The forensic chemistry of alkaloids. The alkaloids: chemistry and physiology. 12: 514-589, (1970)

[7] Fakuruddin M, Alan K M A, Mazumdar R M, Islam S, Nipa MN, Iqbal A and Bhuiyan HR., Anti-bacterial activity of the extract of *Terminaliaarjuna* against multi antibiotic resistant *Vibrio cholera*. Journal of Scientific Research, 3 (1): 129-137, (2011)

[8] Harborne JB., Phytochemical Methods. A Guide to modern techniques of plant analysis. London NewYork. Chapman and Hall. 1988

[9] Joshi AB, Aswathi M and Maya B., *Terminalia tomentosa* Roxb (ex DC) Wght & Arn: Phytochemical investigation. American journal of advanced drug delivery, 1 (3): 224-231, (2013)

[10] Kandil FE and Nassar MI., A tannin, anti-cancer promoter from *Terminalia arjuna*. Phytochemistry, 47 (8): 1567-68, (1998)

[11] Kiew R, and Baas P., *Nyctonthes* is a member of the Oleaceae. Proc. Indian Acad. Sci. (Plant Sci.), 93:349-358, (1984)

[12] Mason TL, Wasseman BP., Inactivation of red beet betaglucan synthase by native and oxidized phenolic compounds. Phytochemistry, 26: 2197-02, (1987)

[13] Meriga B, Naidu PB, Muniswamy G, Kumar GH, Naik RR, and Pothani S., Ethanolic fraction of *Terminalia tomentosa* attenuates biochemical and physiological derangements in diet induced obese rat model by regulating key lipid metabolizing enzymes and adipokines. Phcog Mag, 13: 385 – 92, (2017)

[14] Pettir GR, Hoard MS, Doubek DL, Schmidr JM, Pettir RK, Tackerr LP *et al.,* Antineoplastic agents 338. The cancer cell growth inhibitory. Constituents of *Terminalia arjuna* (Combretaceae). J. Ethnopharmacol, 53 (2): 57-63, (1996)

[15] Rehaman Omike., Phytochemical screening tests and medicinal values of plants active properties, 2010

[16] Row LR andRao GSRS., Chemistry of *Terminalia* species-VI. The constitution of Tomentosic acid, a new triperpene carboxylic acid from *Terminalia tomentosa* wight et arn. Tetrahedron, 18: 827-38, (1962)

[17] Sangetha SN, Zuraini Z, Sasidharan S and Suryani S., Antimicrobial activities of *Cassia surattensis & Cassia fistula.* J. of Mol. Biol. And Biotech, 1: 1-4, (2008)

[18] Sharad V and Tanya G., Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of *Moringa oleifera* Lam. From Rajathan, India. Physiology and Mol Bio of Plants, 23 (1): 239-248, (2017)

[19] Shibata S., Saponins with biological and pharmacological activity. New natural products and plant drugs with pharmacological, biological or

therpeutical activity. Proceedings in Life Sciences, 177-196, (1977)

[20] Shivaji K., Comparative Pharmacognistical studies on different taxa used as an ayurvedic drug Arjuna. Ph.D Thesis, Sri Venkateswara University, (2013)

[21] Singh AP., Controversial herbal drugs of Ayurveda. Scientific publishers, 42-43, (2013)

[22] Srivastava SK, Srivastava SD and Chouksey BK., Antifungal constituents from *Terminalia alata.* Fitoteapia, 72: 106-112, (2001)

[23] Sukalyani D, Diganta D, Sudiopta H, Subhalakshmi G, Ratnamala R and Bansri H., Anti-bacterial and antifungal activity of *Terminalia arjuna* Wight & Arm. bark against multi-drug resistant clinical isolates. Journal of Coastal Life Medicine, 1 (4): 312-318, (2013)

[24] Tschesche R and Wulff G., Antimicrobial action of saponins. Zeitschrift fűr naturfor Schung, 206 (6): 543-546, (1965)

[25] Tschesche R., Pharmacognosy and Phytochemistry (H. wagner & L Horhammer, ed), 274, (1971)

[26] Udaysing Haripatil and Dattatraya K Gaikwad., Phytochemical evaluation and bactericidal potential of *Terminalia arjuna* stem bark. International Journal of Pharmaceutical Sciences and Research, 2 (3): 614-619, (2011)

[27] Visweswari G, Christpher R and Rajendra W., Phytochemical screening of active secondary metabolite present in *Withania somnifera* root: Role in traditional medicine. Int J Pharm Sci Res, 4 (7): 2771-2776, (2013)