Trends in Life Sciences
An International Peer-reviewed Journal
www.sciencejournal.in

PHYTOCHEMICAL SCREENING AND EVALUATION FOR ANTIBACTERIAL ACTIVITY OF *SEMECARPUS ANACARDIUM* L. ROOT EXTRACTS.

Solankar, B. M., Kadam, R. M. and Mulani R. M.
School of Life Sciences, S.R.T.M. University, Nanded, (M.S.), India.
Dept. of Botany, Mahatma Gandhi Mahavidhyalaya, Ahmedpur Dist. Latur, (M.S.), India.
Email ID:solankarbhim85@gmail.com, rmk76@rediffmail.com, mulani1961@gmail.com

ABSTRACT
The plant contains valuable secondary metabolites. In present investigation aqueous extract (Aq. E.) and ethanolic extract (Et. E.) root of *Semecarpus anacardium* L. is selected for phytochemicals and antimicrobial screening. The main purpose of the research work is to check the presence or absence of phytochemicals and antibacterial activity. The secondary metabolites like alkaloids, carbohydrates, proteins, flavonoids, glycosides, triterpenoids, saponins, steroids, starch and tannins were present, resin was absent. The maximum zone of inhibition was observed 18 mm diameter against *Escherichia coli* in root ethanolic extract (Et. E.). The minimum zone of inhibition was observed 07mm diameter against *Staphylococcus aureus* in root aqueous extract of *S. anacardium* L. Ethanolic extract highest antibacterial potential as compared to aqueous extract (Aq. E.) Both extract shown maximum zone of inhibition against gram $-^{ve}$ bacteria as compared to gram $+^{ve}$ bacteria.

KEYWORDS: antimicrobial activity, Phytochemicals screening, *Semecarpus anacardium* L.

INTRODUCTION

Plants are the source of large amount of drugs that are used in treatment of several diseases. The *Semecarpus anacardium* Linn. is an important medicinal plant member of Anacardiaceae family. It is a dry deciduous tree distributed a sub Himalayan tract and in tropical part of India. The plant is used for treatment of various ailments like rheumatism, asthma, epilepsy, nervous debility and also tumours (Ambasta, 1986). Nut oil obtained from nut of *S. anacardium* by burning nut for consumption. Few drops of pericarp oil mixed in milk or ghee or vegetable oil were consumed for treating asthma, weakness. It is also applied on foot to eliminate pain. The dose intake was at random and indiscriminate (Choudhari and Deshmukh, 2006). The pericarp oil contains flavonoids, phenols like catechol and anacardic acid as active constituents (Kirtikar and Basu, 1975).

Botanical description:

Semecarpus anacardium is medium sized dry deciduous tree with a rough bark, yielding acrid juice. **Root:** taproot**,** long grow deep in to the soil. **Stem:** erect, strong, woody with rough bark. **Leaves:** large, crowded, oblong or obvate-oblong, rounded at the apex rounded coriacious,. **Pedicel:** is shorter than the leaves. **Flowers:** small, greenish white sub-sessile, **Calyx:** is about 1 mm. Long,. **Corolla:** petals 4-5 mm. Long, ovate, acute,. **Ovary**: is subglobose densely pilose, crowned with 3 styles.. **Drupe:** size 2.15-4.5 cm. Long, kindly-shaped, obliquely ovoid, smooth shining black when ripe Naik, (1998), Yadav and Sardesai, (2002).

Fruit of *S. anacardium* is divided in two part i.e. hypocarp (fulbibba) and pericarp (nut, seed, and drupe). The hypocarp is reddish yellow colour, bell shape and edible part of fruit. The pericarp is usually obliquely ovoid, spherical and elongated shape and blackish brown and smooth shining colour. The pericarp is divided in to epicarp, mesocarp and endocarp (godambi). *S. anacardium* nut oil is used from sophisticated mark on cloth and mark on nail at election time so it is commonly called as marking nut. Nut is widely used in Indian traditional medicine 'Ayurveda' for the treatment of rheumatoid arthritis, gout and other inflammatory diseases, tumours, asthma, epilepsy psoriasis and leprosy (Khare, 2007). In present study investigation carried out for screening of phytochemicals for antibacterial activity of *S. anacardium* L.

MATERIALS AND METHODS

Collection of sample:

The roots of *Semecarpus anacardium* were collected from Nandu Kachale 18° 49' 12 N, 077° 04 ' 43 E at: Wanwadi Tq: Hadgoan in Nanded district of Maharashtra. The entire twig of *S. anacardium* L. was pressed for preparation of herbarium. The pressed plant was then transferred on the standard herbarium sheet and the identification

was confirmed by Dr. R. M. Mulani and the identified herbarium sheet is preserved in herbarium at School of Life Sciences in Swami Ramanand Teerth Marathwada University, Nanded. Roots of *S. anacardium* L. family Anacardiaceae were collected from farm of Nandu Kachale at Wanwadi Tq: Hadgoan in Nanded district of Marathwada region in Maharashtra State, India..

Shade dried: The roots were cut, air-dried and powdered with grinding machine and stored in plastic container. 25gm powder in 250 ml aqueous and ethanolic solvents for extraction. Vijayalakshmi *et. al.,* (2012)**.** The extraction was done by Soxhlet extraction techniques till dark colouration of the solvent and discolouration of powder extract. The solvents were evaporated to complete dryness by rotavator and stored in eppendorf˜s tube at 4˚C for further use Hassan *et al.*, (2014); Das *et al.,* (2014).

Preliminary qualitative phytochemical screening: The roots of *S. anacardium* L. were extracted with aqueous and ethanolic solvent. The various qualitative tests were undertaken for identification secondary metabolites using various method suggested by Harborne, (1973); Gomathi *et.al,* (2013); Santanu *et al.* (2011); Ali (2000); Jerald and Jerald, (2007) and Wankhade and Mulani (2015).

Bacterial strains: In present investigation two gram $-^{ve}$ and 02 gram $+^{ve}$ bacteria are selected for antibacterial screening namely, *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Bacilus subtilus.* These cultures were obtained from School of Life Sciences, S.R.T.M. University, Nanded. All bacterial strains were subcultured frequently 15 days carried on nutrient agar slant. These slants were stored in $4^0{}_C$ at refrigerator.

Preparation of media: Accurately 13gm nutrient broth was dissolved in 1000ml of distilled water, 25gm agar powder was added. Then these medium was sterilized in autoclaved under 15 Lb for 15 min. About 20ml sterilized semisolid nutrient agar media was poured in to pre-sterilized glass petriplates. It is carried out under aseptic condition in laminar air flow. Then these plates were cool at room temperature for solidified the media.

Antibacterial activity:- The antibacterial activity of aqueous (Aq. E.) and ethanolic (Et. E.) root extracts of *S. anacardium* L. was done by using agar well method which is described by Perez *et. al* (1990). The extract were reconstituted in 10% v/v aqueous dimethyl sulfoxide (DMSO) at concentration of 300mg/ml, 200mg/ml and 100mg/ml. Each nutrient agar plate were inoculated with 0.1ml suspension of 24 hours bacteria old active culture was spreaded with L shaped spreader. In each plate three well of 6mm diameter were made at equal distances using sterile cork borer, 0.1ml of extract was added to each well. The petridishes were incubated at 37^0C for 24 hours. The zone of inhibition were measured by using a standard measuring scale.

RESULTS AND DISCUSSION

In present investigation phytochemical screening of aqueous extract (Aq. E.) and ethanolic extract (Et. E.), root of *S. anacardium* L. have the secondary metabolites like alkaloids, carbohydrates, proteins, flavonoids, glycosides, triterpenoids, saponins, steroids, starch and tannins are present and resin is absent in root ethanolic extract. The highest phytochemicals was observed in ethanolic extract as compared to aqueous extract represented in (Table no. 01). These both extract tested for antibacterial activity, ethanolic extract showed greater antibacterial potential is followed by aqueous root extract of *S. anacardium* L. represented in (Table 02). The maximum zone of inhibition against *Escherichia coli* is 18 mm, 16 mm, 13 mm diameter respectively at concentration 300mg/ml, 200mg/ml and 100mg/ml of root ethanolic extract. It is followed by 10mm, 09mm, 07mm diameter respectively at concentration, 300mg/ml, 200mg/ml and 100mg/ml root aqueous extract of *S. anacardium* L. The ethanolic root extract antibacterial activity against *Escherichia coli, Salmonella typhi, Staphylococcus aureus and Bacillus subtilus* but aqueous root extract only against gram $-^{ve}$ bacteria like *Escherichia coli* and *Salmonella typhi*.

Mohanta, *et. al*. (2007), carried out the experiment for aqueous nut extract of *Semecarpus anacardium* L. for phytochemical and antimicrobial screening. The aqueous and petroleum ether extract observed zone of inhibition against *Staphylococcus aureus* 10 mm and *Sigella flexneri* is 16mm diameter at concentration of 100 mg/ ml. While phytochemical analysis of oil shown the presence of alkaloids, tannins, flavonoids and anthraquinones. Hassan *et. al.* (2009), worked on antibacterial activity by disc diffusion method. The root chloroform extract of *Polygonum hydropiper* maximum zone of inhibition were observed against *Bacilus subtilus* 25.6 mm diameter is followed by

Enterobacter aerogenes 25.3mm diameter at concentration of 300mg/disc. The lowest activity of plant extract against *Salmonela typhi* 13.0 mm diameter zone of inhibition at the concentration of 150mg/disc. Devendra *et. al* (2011) were carried out experiment for antibacterial activity of leaves chloroform extract of *Moringa oleifera* showed maximum zone of inhibition against gram –ve bacteria like *Pseudomonas aeruginosa* were 9.5±mm diameter is followed by *Escherichia coli* 8.8±1.0 mm. diameter. The lowest zone of inhibition against gram +ve bacteria *Staphylococcus aureus* was 6.2±07 mm diameter.

Table 1: Phytochemical screening of aqueous (Aq. E) and ethanolic (Et. E.) root extracts of *Semecarpus anacardium* L.

Sr. no.	Secondary metabolites	test	Root aqueous extract	Root ethanolic extract
1	Alkaloids	Hager's	+	+
		Wagner's	+	+
		Mayer's	+	+
2	Carbohydrates	Anthron	-	-
		Fehling's	-	+
		Molisch's	+	+
3	Proteins	Biuret's	+	+
4	Flavonoids	Shinoda's	-	+
5	Glycosides	Molisch's	-	+
6	Triterpenoids	Liebermann- Burchard's	-	+
7	Resins	Resin	-	-
8	Saponins	Saponin	-	+
9	Steroids	Liebermann- Burchard's	-	-
		Salkowski reaction	-	+
10	Tannins	Tannin's	+	+
11	Starch	Starch	-	+

Where: + = Present and - = Absent.

Table 2: Evaluation of antimicrobial activity of aqueous (Aq. E) and ethanolic (Et. E.) root extracts of *Semecarpus anacardium* L.

Sr. no.	Name of pathogen	Zone of inhibition (mm)							
		Aqueous Extract (mg/ml)			Ethanolic extract (mg/ml)			_ve Control (10% DMSO)	+ve Control Streptomycin. 10mg/ml
		100	**200**	**300**	**100**	**200**	**300**		
01	*Escherichia coli*	07	09	10	13	16	18	-	22
02	*Salmonella typhi*	07	08	09	11	13	14	-	18
03	*Staphylococcus aureus*	-	-	-	07	10	11	-	13
04	*Bacillus subtilus*	-	-	-	-	07	10	-	11

The *Streptococcus pyogenes* was 7.0±0.5 mm diameter. It is reported that maximum zone of inhibition against gram –ve bacteria as compared to against gram +ve bacteria. In present investigation same result ethanolic and aqueous root extract of *S. anacardium.* Dahake *et. al* (2009) worked on antimicrobial activity of ethanolic and petroleum ether leaf extract of *Anacardium occidentale*. The highest zone of inhibition was observed in ethanolic extract against *Staphylococcus aureus* 20mm diameter and *Bacilus subtilus* 19mm diameter. In ethanolic extract highest zone against gram +ve bacteria as compared to against gram –ve bacteria. This test is apposite related to root ethanolic extract of *Semecarpus anacardium* L. Thomas *et. al* (2012) carried out work on antibacterial activities leaves and bark aqueous

and ethanolic extracts of *Anacardium occidentale.* The highest zone of inhibition showed in *Escherichia coli* 12±0.90mm diameter. The lowest zone of inhibition showed *Enterococcus faecalis* 7±0.00 diameter. Sharma *et. al* 2010) work on antibacterial activities efficacy nut oil of *Semecarpus anacardium* L. against gram +ve bacteria is *Bacillus subtilus, Staphylococcus aureus,* and gram –ve *Proteus vulgaris, Escherichia coli* etc. In this *Staphylococcus aureus* showed highest zone of inhibition 16.7±0.80 mm diameter.

CONCLUSION

In present investigation study of phytochemical screening of aqueous (Aq. E.) and ethanolic (Et. E.) root extracts of *Semecarpus anacardium* L. It's find out secondary metabolites like alkaloids, carbohydrates, proteins, flavonoids, glycosides, triterpenoids, saponins, steroid's and tannin's. These secondary metabolites showed antimicrobial activity against gram -ve bacteria as compared to gram $+^{ve}$ bacteria. It is concluded that root ethanolic extract contains highest phytochemicals. It showed maximum zone of inhibition as compared root aqueous (Aq. E.) extract. This is confirmed that this investigation can be used in the folk medicine in source of antibacterial treatment of many diseases.

ACKNOWLEDGEMENT

Authors are thankful to Director School of Life Sciences for providing necessary facilities. We are extremely thankful to Rajiv Gandhi Science and Technology Commission (RGSTC) Government of Maharashtra for financial support.

REFERENCES

Ali M. (200). Textbook of Pharmacognosy. CBS Publishers and Distribution Pvt. Ltd. New Delhi.

Ambasta S. P. (1986). The useful plant of India. *Counc. Sci. Ind. Res Publication New Delhi* pp. 566.

Bagewadi Z. K., Siddanagouda R. S. and Baligar P. G. (2012). Phytochemical screening and evaluation of antimicrobial activity of *Semecarpus anacardium* L. nuts. *Int. J. Pharmacol. Pharmaceut. Tech.* 1 (2): 68-74.

Banu K. S. and Cathrine L. (2015). General techniques involved in phytochemical analysis. *Int. J. Advanced Res. Chem. Sci.* 2(4): 25-32.

Biradar S. R., Birajdar D. S. and Rachetti B. D. (2015). Phytoconstituent of medicinally important plant *Andrographis paniculata* Nees. *Int. J. Pharmacog.* 2 (1): 55-58.

Choudhari, C. V. and Deshmukh, P. B. (2006). Bhilwa-a popular medicine. ***International Conference on Ethno Pharmacology and Alternative medicine* (ICEAM-06) at Amla Cancer Research Center, Trissur, Kerala, during 20-22 jan.2006. Proceeding PP**.116.

Dahake, A. P., Joshi, V. D. and Joshi, A. B. (2009). Antimicrobial screening of different extract of *Anacardium occidentale* Linn. leaves. ***International journal ofChem Tech. Research*. Vol. 1 (4):**856-858.

Das N., Islam M. E., Jahan N. Islam., M. S., Khan, A., Islam M. R. and Pravin M. S. (2014). Antioxidant activities of ethanolic extracts and fractions of *Crescentia cujete* leaves and stem bark and the involvement of phenolic compounds. *BMC Complementory Alternative Medicine*. 14-45.

Devendra B. N. (2011). Antimicrobial activity of *Moringa oleifera* Lam. Leaf extract against selected bacterial and fungal strains. *Int. J. Pharma Biosci.* 02 (3): 13-18.

Gomathi R., Priyadarshhini T., Kirithika T. and Usha K. (2013). Phytochemical screening and free radical scavenging activity of *Medicago sativa* leaves. IJPRBS, **2(4):** 29-38.

Harborne J. B. (1998). *Phytochemical method- A guide to modern technique of plant analysis. 3rd ed*. New Delhi: Spring (India) Private limited. 203-208.

Hasan M. F., Das R., Khan A., Hossain M. S. and Rahman M. (2009). The determination of antibacterial and antifungal activities of *Polygonum hydropiper* (L.) root extract. *Advances Biol. Res.* 3 (1-2): 53-56.

Hassan L. E., Ahmed M. K., Baharetha H. M., Muslim N. S., Nassar Z. D. and Majid A. A. (2014). Correlation of antiangiogenic, antioxidant and cytotoxic activities of some sudanese medicinal plants with phenolic and flavonoids contents. *BMC Complementory Alternative Medicine.* 14-406.

Ilanchenzhian R., Acharya R. N., Roshy Joshep C., Harisha C. R. (2012). Selection of Bhalataka *(Semecarpus anacardium* Linn.) fruits for therapeutic uses- a scientific approach. *Ayurpharm Int. J. Ayur Alli. Sci.* 1 (5): 90-98.

Ilanchezhian R., Roshy Joseph C., Acharya R. N., Harisha C. R. and Shukla V. J. (2011). Pharmacognostical and physiochemical analysis of Bhallataka (*Semecarpus anacardium* Linn.)- Fruit. *Phcog. J.* 3 (20): 9-16.

Jerald E. E. and Jerald E. E (2007). *Text book of pharmacognosy and phytochemistry*, CBS publisher and distributers. New Delhi.

Joshi B., Lekhak S. and Sharma A. (2009). Antibacterial property of different medicinal plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum* and *Origanum majorana*. *J. Sci.Engineering Tech.* 5 (1): 143-150.
Kaladhar DSVGK, Duddukuri G. R. and Yarla N. S. (2014). Phytochemical analysis, antioxidant and antimicrobial activities from raw fruits peel crude extracts of *Annona squamosa* Linn. *World J. Pharmacy Pharmaceut. Sci.* 4 (1): 1373-1380.
Kalase, V. and Jadhav V. (2013). Preliminary phytochemical and antibacterial studies on leaf and bark of *Holigarna grahamii* (Wight) Kurz. (Anacardiaceae). *J. Applied Pharmaceut. Sci.* 3(06): 111-113.
Kantamereddi VSSN., Lakshmi Y. N. and Kapsu VVVS. (2010). Preliminary phytochemical analysis of some an important Indian medicnal plant species. *Indian J. Pharmacy Biol. Sci.* 1(4): 351-358.
Khare C. P. (2007). Indian medicinal plants Illustrated dictionary. *New Yark: Springer science business media* LLC; 597.
Kirtikar K.R. and Basu B.D. (1975). *Indian Medicinal Plants.*1-600
Mohanta T. K., Patra J. K., Ratha S. K., Pal D.K. and Thatoi H. N. (2007). Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semecarpus anacardium* L. *Scientific Res. Essa.* 2(11): 486-490.
Mulani R. M., Nandedkar, P. H. and Solankar B. M. (2013). Phytochemical estimation and antimicrobial activity of *Cordia dichotoma* F. *Asian J. Phytomed. Clin. Res.* 1(4): 211-217.
Mulani R. M., Solankar B. M. and Surwase B. S. (2014). Examining the effect of seed size and weight on seed germination of *Semecarpus anacardium* L. - A case study of Wanwadi germplasm, dist. Nanded, Maharashtra. *J. Global Biosci.* 3 (7): 1084-1088.
Naik V. N. (1998). Flora of Marathwada, Vol. I and II, *Amrut Prakashan Aurangabad.*
Perez C., Paul M. and Bazerque P. (1990). Antibiotic assay by agar-well diffusion method. *Acta Biol. Med. Exp.* 15: 113-115.
Pednekar P. A. and Raman B. (2012). Pharmacognostic and phytochemical studies of *Semecarpus anacardium* (Linn). f. leaves *Int. J. Pharmacy Pharmaceut. Sci.* 4(3): 682-685.
Pednekar P. A. and Raman B. (2013). Assessment of *Semecarpus anacardium* (Linn). f. leaf methanolic extract for their antibacterial, antifungal and antioxidant activity. *Int. J. Pharmacy Pharmaceut. Sci.* 5 (1): 170-174.
Pradeep D. P. and Saj O. P. (2010). Phytochemical and antibacterial studies of leaf and bark of extracts of *Holigarna arnottiana* Hook f. *J. Pure and App. Micro.* 4(1):139-148.
Ravishankar B. and Shukla V. J. (2007). Indian system of medicines: A Brief Profile. *African J. Traditional, Complimentary Alternative Medicines.* 4(3): 319-337.
Santanu S. Subrahmanyamc E. V. S., Chandrashekar K. and Shashidhara C. S. (2011). Isolation and characterization of triterpenoids and fatty acid ester of triterpenoids from leaves of *Bauhinia variegate*. *Der. Pharma. Chemical.*. 3(4): 28-37.
Sharma A., Barman, N. and Malwal M. (2010). Antimicrobial efficacy of nut oils of *Semecarpus anacardium* L. f. a marking nut tree. *Biotech.* 9 (3): 383-386.
Singh A. P. (2005). Promising phytochemical from Indian medicinal plants. *Ethnobotanical Leaflets.* 9: 15-23.
Thomas B. T., Kesi C., James E. S. and Musa O. S. (2012). Antibacterial and antiinflammatory activities of *Anacardium occidentale* leaves and bark extracts *Crown J. Medicine.* 2 (2): 20-24.
Vijayalakshmi A., Ravichandiran V., Malarkodi V., Nirmala S. and Jayakumari S. (2012). Screening of flavonoids "quercetin" from the rhizome of *Smilax china* Linn. for anti-psoriatic activity, *Asian Pacific J. Tropical Biomed.* 2 (4): 269-275.
Wankhade M. S. (2016). "Digitization and chemoprofiling (flavonoids) of selected trees belonging to family Leguminosae from Kinwat and Mahur forest ranges of Nanded district in Maharashtra" Ph. D. Thesis of SRTMU, Nanded.
Wankhade M. S. and Mulani R. M. (2015). Chromatographic fingerprint profiling and phytochemical on leaf and bark methanolic extract of *Ougeinia oojeinensis* (Roxb). Hochr. *Int. J. Curr. Res.* 7 (2): 12665-12673.
Wankhade M. S. and Mulani R. M. (2015). Preliminary phytochemical screening and chromatographic fingerprint analysis of *Acacia leucopholea* Roxb. Methanolic leaf and bark extract by HPLC techniques. *Indo American J. Pharmaceut. Res.*. 1(4): 184-191.
Yadav S. R. and Sardesai M. M. (2002). Flora of Kolhapur district. Shivaji university of Kolhapur pp. 129.

