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BIOLOGICAL EVALUATION OF SHWETA &KRISHNA NIRGUNDI, VITEX SPECIES WITH DIVERSE MEDICINAL QUALITY

Ayurveda

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ABSTRACT

Nirgundi a medicinal shrub available in two colour variants ie *Krishna (Vitex negundo var. purpurascence)* and *Shweta(Vitex negundom var. negundo)*. Medicinally this is said to be best antimicrobial and analgesic.

Materials and Methods: Matured aerial parts of these two plants were collected and evaluated for macro-microscopic, physicochemical, phytochemical and antimicrobial activity.

Results: Microscopic and physico-chemical study of these two test drugs has not shown any variation. Phytochemical study revealed Saponins exclusively at *Vitex negundom var. negundo.* Methanloic extract of two types of test drugs have shown zone of inhibition for both samples against *S. aureus, S. Pyogenes, P. aeruginosa, E. coli.*

KEYWORDS

Krishna Vitex negundo var. purpurascence , Shweta Vitex negundom var. negundo, Microscopic, physico-chemical, zone of inhibition

INTRODUCTION:

Nirgundi an offensive medicinal shrub with divine medicinal property found commonly at hedges, roadsides[1]. The literal word of *Nirgundi* is which gives protection against diseases[2]. Simultaneously the '*Guda Dhatu'* literally means which does not need any protection[3]. Because of the potent active principle in the form of secondary metabolite, it may not need any special care for its growth. Description of this plant is available in all major textbooks of *Ayurveda.*Commonly we will get purple and white variety of these plants in nature. *Ayurveda* treatises mentioned them as *Krishna (Vitex negundo var. purpurascence)* and *Shweta(Vitex negundom var. negundo)* as two types of *Nirgundi*[4].

It is a large deciduous shrub, growing up to 6m tall, with thin grey smooth bark and strong heart wood with pale blue to purplish black flowers[5]. The *Krishna Nirgundi((Vitex negundo var. purpurascence)* with more purplish tinge over whole plant and specially on inflorescence[6]. Plant found world-wide and India found along waysides and near waste courses and frequently planted along roadsides[7].

Charaka samhita makes reference of both these varieties of *Nirgundi* advices it to use as *Krimighna*(antibacterial), *Vishaghna*(Antidote)[8]. *Sushruta samhita* advices the oil prepared by using the root and leaves of *Krishna Nirgundi*(*Vitex negundo var. purpurascence)* in various pathological conditions like *Vatavyaadhi, Kushta, Paama, Apachi*[9] .

Kaideva nighnatu(a lexicon) mentions Neela Nirgundi, and he gives the synonyms like *Neelapushpa, Krishna, Neela manjari, Vanja*[10]. *Bhavaprakasha* (a lexicon) mentions clearly the plant with *Neela pushpa* is *Neela Nirgundi* for *(Vitex negundo var. purpurascence)* he recommends it as *Keshya*(Hair toner)[11].

Traditional healers, recommends this plants as insecticide, mosquito repellent, as a preservative and recommends the usage of blue flowered variety as analgesic remedy[12]. Colour variation in plants is due to anthocyanin content a glycoside present in cell sap which impart red, purple, blue, violet colour for plant or plant parts[13].

Hence with all these background a study has been designed to evaluate biological differences between these two varieties of Nirgundi ie *Krishna (Vitex negundo var. purpurascence)* and *Shweta(Vitex negundom var. negundo).* A detailed comparative Macro-microscopic, physico-chemical, phytochemical and antimicrobial activity study has been planned for these two samples as per standard methodology.

MATERIALS AND METHODS:

Matured aerial parts of *Krishna (Vitex negundo var. purpurascence)* and *Shweta(Vitex negundom var. negundo)*were collected from their natural habitat, authenticated using flora and botanist opinion. Sample deposited in Pharmacognosy department of SDM centre for Research in Ayurveda and Allied sciences. Macroscopic views of all parts of samples were taken along with their organoleptic records. Few samples kept in FAA solution for Microscopy and rest shade dried, powder prepared and used for further study[14].

MICROSCOPY

The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars[15].

Physicochemical study:

Physico-chemical standards of seed like loss on drying at 105°C, total Ash, acid insoluble ash, water soluble ash, alcohol soluble extractive & water soluble extractive were detected as per standard methodology.[16]

PHYTOCHEMICAL STUDY:

Test sample powder evaluated for preliminary phytochemical screening for detection of various phytoconstituents like alkaloids, carbohydrates, steroids, saponins, tannins, flavonoids, phenol, coumarins, triterpenoids, carboxylic acid, resin & quinine.[17]

Antimicrobial study:

Plant extract preparation

10gm of dried plant powder was extracted with 100ml of methanol kept on a rotary shaker for 24 hour. Thereafter, it was filtered and centrifuged at 5000gm for 15 minutes. The supernatant was collected and the solvent was evaporated to make the final volume 1/5 of the original volume. It was stored at 4° C in airtight bottles for further studies[18].

MEDIA USED FOR THE STUDY

Muller-Hinton agar, Sheepblood agar, Peptone water, Todd Hevit broth

Microorganisms tested

Staphylococcus aureus(ATCC 25923)
Streptococcus pyogenes(ATCC 25924)
Escherichia coli(ATCC 25922)
Pseudomonas aeruginosa (ATCC 27853)

METHODOLOGY

Antimicrobial susceptibility was tested on Muller-Hinton agar for *Staphylococcus aureus,Escherichia coli* and *Pseudomonas aeruiginosa*, while *Streptococcus pyogenes* was tested on sheep blood agar. The broth suspension of the organism was prepared. These were inoculated in respective media by lawn culture or carpet culture. A sterile borer was used to punch wells in the inoculation media(per plate 5 wells). In each well 100µlitre of the plant extract was placed in each well. Then standard control discs were put in the centre of the plate, to test the efficacy of the procedure. *Ampicillin* disc used as standard for *Staphylococcus aureus* and *Streptococcus pyogenes*, while *Gentamycin* for *Escherichia coli* and *Pseudomonas aeruginosa*. The plates were further incubated overnight at 370c. The inhibition zone

was recorded (in mm) after overnight incubation using a transparent scale.

Mean inhibition zone size (average of 5 wells) of each test solution against each particular microorganisms (S. aureus, S. Pyogenes, P. aeruginosa, E. coli, were recorded and results were analyzed statistically.

RESULTS:

Macroscopy:

Upper aerial parts of both samples *Krishna (Vitex negundo var. purpurascence)* and *Shweta(Vitex negundom var. negundo)*3-5 floiate, entire, dark green above, pale beneath. Lateral cyme with bluish purple florets. *Krishna (Vitex negundo var. purpurascence)*will have purplish tinge over all parts. Both are aromatic, pungent.

MICROSCOPY:

Krishna (Vitex negundo var. purpurascence) : TS of leaf portion at petiole show the presence of lenticels at both lower and upper epidermal region. Vascular bundle both xylem and phloem seen at central region. Leaf lamina possess Rossette crystals of calcium oxalate.

Shweta(Vitex negundom var. negundo): Secretary cells found at leaf lamina portion.TS of stem show the presence of epidermis, hypodermis, cortical region, xylem and phloem elements.

Figure 1 a Macroscopic view

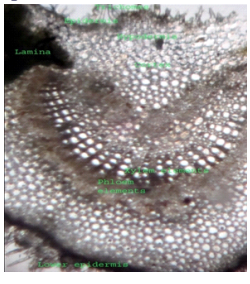


Figure 1 b. TS of leaf petiole

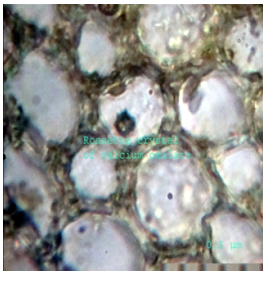


Fig 1 c. Rossette crystals of calcium oxalate in leaf lamina

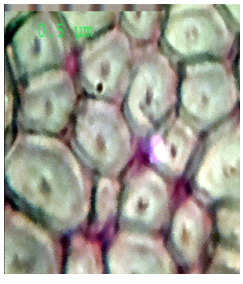
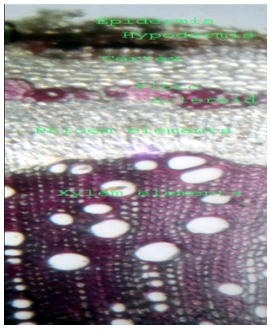


Fig 1 d Non lignified fibres at TS stem

Figure 1. Macro-microscopy of *Krishna (Vitex negundo var. purpurascence)*

Figure 2 a Macroscopic view Figure



2b. Outline of TS of stem

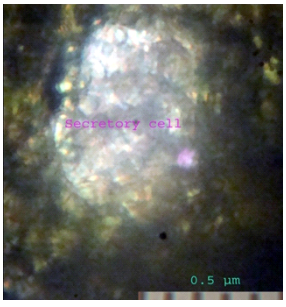


Fig 2 c .Secretory cell in leaf lamina

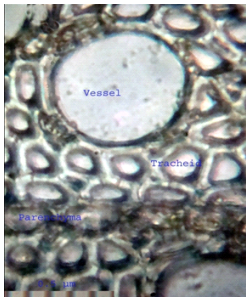


Fig 2 d. Xylem elements in stem

Figure 2. Macro-microscopy of *Shweta(Vitex negundom var. negundo)*

Physico chemical standards

Loss on drying, Ash value, Acid insoluble ash, water and alchohol soluble extractive value of both are displayed in table 1. There is not much difference among two samples.

Table 1 Physicochemical standard:

Physico chemical standards	*(Vitex negundo var. purpurascence)*	*Vitex negundom var. negundo*
Loss on drying	4.8	4.5
Ash value	6.8	6.3
Acid insoluble ash	1.3	1.5
Water soluble extractive	9	8
Alchohol soluble extractive	8	7

Phytochemical study

Secondary metabolites are the resultant of plant growth as per their genetic structure, ecology and many other factors. Table 2 displays the presence of these factors among two samples. Saponins are exclusively found at *Vitex negundom var. negundo.* Both sample sho the presence of protien, carbohydrate, tannin, flavanoids and triterpenoids.

Table 2 Phytochemical test:

Secondary metabolites	*Vitex negundo var. purpurascence*	*Vitex negundo var. negundo*
Protien	+	+
Carbohydrates	+	+
Tanins	+	+
Saponins	-	+
Flavanoids	+	+
Phenols	-	-
Alkaloids	-	-
Triterpenoids	+	+

Antimicrobial study:

Methanloic extract of two types of test drugs were studied for antimicrobial activity. Mean inhibition zone size (average of 5 wells) of each test solution against each particular microorganisms (*S. aureus, S. Pyogenes, P. aeruginosa, E. coli.)* were recorded and results displayed in Table 3 .There was not any marked change among zone of inhibition between two drugs.

Table 3 Antimicrobial activity data on Methanolic extract of *Krishna (Vitex negundo var. purpurascence)* and *Shweta(Vitex negundom var. negundo)*

Plant sample	Zone of inhibition (in mm)											
	S. *aureus*			**S. *pyogenes***			**E. *coli***			**P. *aeruginosa***		
	C	M	T	C	M	T	C	M	T	C	M	T
Vitex negundo var pupurascence	**38**	**18**	**13**	**32**	**21**	**14**	**19**	**14**	**11**	**29**	**18**	**15**
V negundo var. negundo	**38**	**18**	**14**	**30**	**20**	**13**	**19**	**14**	**11**	**30**	**18**	**16**

(C- Control group, M- Methyl alcohol, T- Test group, mm-millimeter)

DISCUSSION AND CONCLUSION

Plants are a source of diverse medicinal potency, with their variant types and subtypes. Nirgundi an offensive shrub with two variants like as *Krishna (Vitex negundo var. purpurascence)* and *Shweta(Vitex negundom var. negundo)* mentioned in Ayurveda treatises with different pharmacological properties. Anthocyanin a glycoside is said to be responsible for colour variation among plant species[19]. Hence with all these questions in mind a study has been designed to evaluate biological difference among these two plants.

Macroscopic variation among these two plants was clearly visible with purplish black tinge over *Krishna (Vitex negundo var. purpurascence).* Microscopic and physico-chemical study of these two test drugs has not shown any variation. Phytochemical study revealed Saponins exclusively at *Vitex negundom var. negundo.* Methanloic extract of two types of test drugs were studied for antimicrobial activity. Antimicrobial susceptibility test has shown zone of inhibition for both samples against *S. aureus, S. Pyogenes, P. aeruginosa, E. coli.* Plant activity is because of its secondary metabolite and depends on many factors like growth, geographical condition, season etc. In this study *Krishna (Vitex negundo var. purpurascence)* and *Shweta(Vitex negundom var. negundo)*have not shown much anatomical or biological variation.

REFERENCES:

1. K Ravikumar et al, Photo Guide to selected medicinal plants of Karnataka; FRLHT, Bangalore India, 2009, pp105
2. Viadya Bapalal; Nighantu Adarsha; Chaukhamaba Bharati Academy; Varanasi, 2009, Vol(II), pp 244.
3. Nishteshwara K, Text book of Dravyaguna; Chaukamba Surabharati Prakshana; Varansai, Ist edition, 2007, pp 293
4. Mallya Suma V, Comprehensive study of Plants in Surasaadi Gana wsr to their antibacterial activity(PhD thesis); Maharashtra University of Health Sciences, Nashik, 2009, pp 117
5. Anonymous. The wealth of India, A dictionary of Indian raw materials & industrial products Vol-X. Newdelhi: Council of scientific & industrial research: 2009 P.522
6. Khare CP, Indian medicinal Plants an illustrated dictionary, Springer: 2007 P. 710
7. Bhat Gopalkrishna K, Flora of Udupi, Indian Naturalist (Regd.); Udupi, Ist edition 2003, pp 507.
8. Hegde Prakash, Harini A, A text book of Dravyaguna Vijnana, Chaukamaba Publications, New Delhi, Vol (II) 2017, pp 615.
9. Sharma Priyavrit, Classical uses of medicinal Plants; Chaukambha Vishwabaharati; Varanasi, 2004 pp 214
10. Sharma PV, Sharam Guruprasad, Kaiyadeva Nighantu; Chaukamabha orientalia, Varanasi, Ist edition, 1979, pp 26.
11. Singh Amrit pal, Bhavaprakasha Nighantu, Chaukambha Orientalia, Varanasi, Ist edition, 2007, pp 96
12. Anita Rani, Sharma Anupam, The genus Vitex; A review; Pharmacognosy reviews, 2013; 7(14)pp188
13. Gangulee, Das and Datta, College Botany, New Central Book Agency(P) Ltd., Vol (I), 6th edition 2002; pp 543.
14. Wallis TE. Textbook of Pharmacognosy. New Delhi: CBS Publisher and Distributors: 1985; P.527
15. Evans WC., Trease and Evan's Pharmacognosy, 15th ed. London: WB Saunders Ltd: 2002; P527-45
16. Anonymous, Quality control methods for medicinal plant materials, Geneva: World Health Organization; 1998; P.25-8
17. Bani Shashikala, Mallya Suma V, Prabhu Suchitra, Quality Control constraint of Guizotia abyssinica Cass, source of medicinally used edible oil seeds, The journal of Phytopharmacology 2018;7(5); 431-36.
18. Mallya Suma V, Nesari Tanuja, Antbacterial activity profile and quality standards of Cymbopogon citrates stapf.- an aromatic grass used in Indian system of medicine; Journal of Ayurvedic and Herbal Medicine 2016;2(3); 63-66.
19. Dutta AC, Botany for Degree Students, Oxford University press; 6th Edn. New Delhi, 2010, pp 127.