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## PHYTOCHEMICAL INVESTIGATION AND BIOLOGICAL ASSESSMENT OF 12-HYDROXY-OCTADEC-CIS-9-ENOIC ACID ISOLATED FROM *SWIETENIA HUMILIS* ZUCC. SEED OIL

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### Keywords:

*Swietenia humilis* Zucc, Ricinoleic acid, Antimicrobial activities, Anthelmintic activity

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**ABSTRACT:** The genus *Swietenia* belongs to Meliaceae family which consists of 37 genera and about 300 species which is found in most of the parts of India. In present investigation we made an efficient effort to extract ricinoleic acid from the seed oil of *Swietenia humilis*, Zucc. They are used in medicine folklore to cure various diseases in Mexico, Africa and India. Physicochemical properties of *Swietenia humilis* Zucc seed oil were identified along with its fatty acid composition. The structure of the ricinoleic acid was confirmed by IR, NMR and Mass spectral studies. The extracted ricinoleic acid was screened for its antimicrobial and anthelmintic activity.

**INTRODUCTION:** Unlike most synthetic drugs that have pure ingredients, such as small molecules or just a single protein, one herb has complex ingredients. Those different ingredients in one herb may balance each other, buffer each other, and act synergistically to make the systemic effect more powerful. Herbs have been used for centuries by people around the world. They may be the oldest "evidence-based medicine". Herbal remedies have been recorded to cure all kinds of diseases for billions of people as a critical therapeutic method in many cultures. The uses of herbal medicine have received great interest in bio-medical research and are important as chemotherapy<sup>1</sup>. Among such plant *Swietenia humilis* belonging to Meliaceae family consists of 37 genera and about 300 species.

It is mainly found on belts of Western Ghats of India. The tree grows up to 30 - 40 feet. The bark contains tannin and may serve as an antipyretic, tonic and astringent<sup>2</sup>. It is used as a substitute for a cinchona bark in the West Indies. The bark is also used in Jamaican tanneries. The timber is known for making furniture, fixtures, inlay, boat, caskets and musical instruments specially drums<sup>3</sup>. The seeds of *Swietenia humilis* are used in traditional medicine to treat chest pains, amoebiasis, coughs, cancer, and also for their anthelmintic properties. The tetranortriterpenoids humilinolide from the *Swietenia humilis* seeds induces smooth muscle (ileal and uterine) contraction<sup>4</sup>. Literature reports that the seed extract of *Swietenia humilis* had *in vitro* antifungal activity for the control of *Rhizopus stolonifer*<sup>5</sup>.

Literature reveals the physicochemical properties of *Swietenia humilis* seed oil were identified along with its fatty acid composition and eight fatty acids were identified as palmitic, stearic, oleic, linoleic, and linolenic acid<sup>6</sup>. However, no reports were found on hydroxy fatty acids. Considering the extensive application of hydroxy fatty acids<sup>7</sup> and

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further in continuation of our studies<sup>8</sup> on hydroxy fatty acids, we have efficiently isolated ricinoleic acid from the seed oil. The structure of the ricinoleic acid was confirmed by IR, NMR and Mass spectral studies. The isolated ricinoleic acid was further screened for its antimicrobial and anthelmintic activity. The ricinoleic acid exhibits a promising biological activity.

## MATERIALS AND METHODS:



SEED OF SWIETENIA HUMILIS ZUCCA

**Plant materials:** Seeds of *Swietenia humilis* plant were collected from the forest region of Dandeli Karnatak and dried for 15 days. Dried seeds were crushed, extracted using successive solvent fractionation extraction using Soxhlet extractor for 24 hours. Solvents used for extraction are diethyl ether and n-hexane.

**Phytochemical test:** The extracted oil was purified and dried by blowing nitrogen gas stream. Various physical tests like refractive index, specific gravity<sup>9</sup>, Iodine value, Reichert-Meiseel valve, saponification valve<sup>10</sup> and chemical test includes Halphen test<sup>11</sup> was performed to know the physical characteristic of oil. Chemical degradation was estimated using standard methods. Thin layer chromatographic investigation involves direct TLC<sup>12-13</sup> and the picric acid TLC<sup>14</sup>.

The analytical TLC was performed on glass plates coated with 0.25 or 1.0 mm layers of silica gel 'G' using 20 or 30 % in hexane as the solvent system. The preparative TLC was affected on 20 cms X 20 cms plates with 1.0 mm layers of silica gel. When the plates were sprayed with dichlorofluorescein acids separated bands were clearly visible under the ultraviolet (UV) light.

The fatty acids from the silica were extracted with ether. Other analytical values of the oil obtained were determined according to the standard method<sup>15-16</sup>.

**Instrumentation:** The UV spectra were taken on a Hitachi 150-20 Model instrument using 0.001% concentration in methanol. The infrared spectra were recorded on an Impact 410 Model Fourier Transform Infrared (FTIR) instrument as liquid films. The NMR spectrum was recorded on a Bruker (300 MHz) instrument using CDCl<sub>3</sub>. The GLC analysis was carried out on a Perkin-Elmer Model Sigma unit using column of 15% DEGS on chromosorb w, 45-60 mesh. The temperatures in the injection port and detector port were 240°C, whereas the oven was programmed at temperature of 190°C. The nitrogen flow was 30 ml/min. The machine recorded directly the weight percent of individual peaks which were identified by comparing their retention times with those of standard reference samples under similar conditions.

## Biological activity:

**Antimicrobial screening:** The antimicrobial activity of the ricinoleic acid was screened by agar well radial diffusion method against bacterial strains belonging to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* and fungal strains *Candida albicans* and *Candida parapsilosis* respectively<sup>17</sup>. The microbial strains were collected from different infectious status of the patients with the help of authorized physicians, in district health centre of Gulbarga, Karnataka state, India. The clinical isolates were identified in Microbiology Laboratory, Gulbarga University following the standard method<sup>18</sup>.

The bacterial and fungal spore suspensions were diluted in 10<sup>-1</sup> to 10<sup>-8</sup> phosphate buffered saline. Samples were homogenized and then loaded in six aliquots of 20 µL each onto nutrient agar plates. The working cultures of bacteria and fungi were prepared by inoculating a loopful of each test microorganism in 3 mL of nutrient broth and potato dextrose broth respectively. Broths were incubated at 37°C and 24°C for 24-48 h. The suspension was diluted with sterile distilled water to obtain approximately 10<sup>6</sup> CFU/mL.

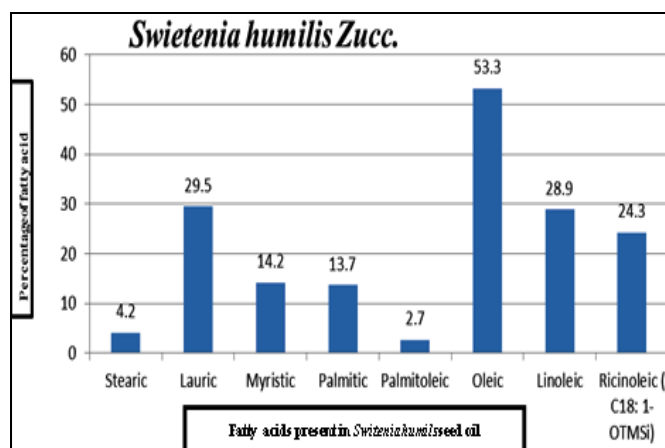
The test compound were dissolved in 5% aqueous dimethyl sulfoxide (DMSO; that enhances compound solubility) to get stock solutions. Commercial bactericide ampicillin and fungicide fluconazole were used as standards (100 µg/100 µL of sterilized distilled water) concomitantly with the test samples.

A sensitive agar well radial diffusion technique<sup>19</sup> was used for the assessment of antimicrobial activity of the test samples. Sterilized nutrient agar medium and potato dextrose agar medium were poured into sterilized petri dishes separately. Nutrient broth containing 100 µL of 24 h incubated bacterial cultures of clinical isolates was spread on the nutrient agar medium. Potato dextrose broth containing 100 µL of agar were incubated for 48 h for fungal cultures of clinical isolates and was spread on the potato dextrose agar medium. Wells were created using a sterilized cork borer in an aseptic condition. 20 µL of test compounds and 100 µL of standard drug ampicillin were loaded on to their corresponding wells of bacterial plates and 20 µL of test compound and 100 µL of standard drug fluconazole were loaded on to their corresponding wells of fungal plates. The bacterial plates were incubated at 37 °C for 24 h and fungal plates were incubated at 24°C for 48 h. The diameter of the zone of complete inhibition of the bacteria and fungi was measured to the nearest around each well and readings were recorded in mm. The results of these experiments are expressed as mean ± S.E.M. of three replicates in each test.

**Anthelmintic activity:** The anthelmintic activity of the ricinoleic acid was evaluated as per the reported method<sup>20</sup>. Twelve groups of animals with three earthworms in each groups, each earthworm were separately released into 20 ml of desired formulation in normal saline, Group I earthworm were released in 20 ml normal saline in a clean petri plate. Group II, III, IV, V, VI, VII and VIII earthworms were released in petri dish containing compound at the dose of 50 mg in 20 ml of normal saline. Group IX earthworms were released in normal saline containing standard drug piperazine citrate (50 mg/ml). Earthworms were observed; the time taken for paralysis and the time taken for death was monitored and documented in minutes. Paralysis time was analyzed based on the behavior of the earthworm with no revival body state in normal saline medium.

Death was concluded based on total loss of motility with faded body color<sup>21</sup>.

**Result and Discussion:** The isolated ricinoleic acid is viscous pale brown oil with molecular formula C<sub>18</sub>H<sub>34</sub>O<sub>3</sub>. B.P: 245°C. *Swietenia humilis* contains saturated fatty acid such as Lauric acid 29.5%, Stearic acid 4.2%, Myristic 14.2%, Palmitic acid 13.7% and unsaturated fatty acids such as Palmitoleic acid 2.7%, Oleic acid 53.3%, Linolenic acid 28.9% and ricinoleic acid 24.3 % as summarized in **Fig. 1**. The presence of hydroxy fatty acid is initially confirmed by various tests like direct TLC test and chemical degradation studies and the results depicted in **Table 1**. Further structure of the ricinoleic acid confirmed by IR, NMR, Mass spectral analysis the results were found to be in good agreement with the predicted structure.



**FIG. 1: GRAPH: COMPOSITION OF SILYLATED METHYL ESTERS OF *SWIETENIA HUMILIS* ZUCC.SEED OIL**

**Spectral data Analysis:** IR ( $V_{max}$  cm<sup>-1</sup>) = 3460 (-OH), 2920 (aliphatic chain), 1630 (C=O), 1455 (C=C). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, δ ppm) 11.2 (1H, s, -COOH), 5.48 (2H, m, CH=CH), 3.25(1H, m, bearing -CHOH), 2.4 (1H,s, -OH D<sub>2</sub>O exchangeable), 2.23(2H,m,CH<sub>2</sub>), 1.96 (2H, m, CH<sub>2</sub>) 1.56 (2H, m, CH<sub>2</sub>), 1.44 (2H, m, CH<sub>2</sub>), 1.33 (4H, m, CH<sub>2</sub>) 1.29(14H, m, CH<sub>2</sub>), 0.96 (3H, t, terminal -CH<sub>3</sub> protons). <sup>13</sup>C NMR spectra: δ171(-COOCH<sub>3</sub>), 50 (-COOCH<sub>3</sub>), 129(Cis, CH=CH), 37 (CH<sub>2</sub>-CH=CH), 81 (-CHOH). Mass spectra (*m/z*, *I*, *rel*, %): 384 (14), 299(8), 270(28), 187(100), 73(45).

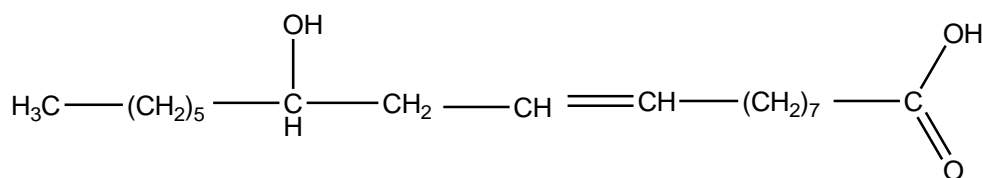
All the analytical data obtained from the above, the structure of the isolated compound found to be ricinoleic acid and the predicted structure is given in the **Fig. 2**.

In the mass spectra, the ion peak at  $m/z$  384 indicates for the presence of carbon chain with the hydroxyl olefinic ester which was identical to the TMSi derivative of authentic methyl ricinoleate. The ions at  $m/z$  187 and 299 results from the alpha

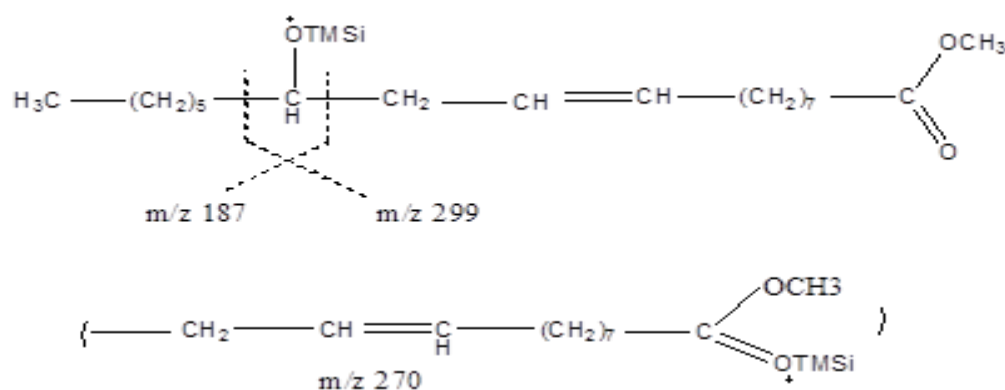
cleavage on both sides of the TMSi group of the unsaturated fatty ester. TMSi rearrangement<sup>22</sup> fragmentation ion at  $m/z$  270 unequivocally established the position of the hydroxyl group at C12 shown in **Fig. 3**.

**TABLE 1: PHYSICO-CHEMICAL TESTS OF SWIETENIA HUMILIS ZUCC. SEED OIL**

Various Test	Seed oil from <i>Swietenia humilis</i> Zucc.
Oil Content in seeds	29.5 %
Hydroxy fatty acid yield	24.3 %
Iodine value (unsaturated)	76.0
Saponification value (greater proportions of higher fatty acids.)	181.0
Direct TLC test (test for hydroxy fatty acid)	Positive
Halphen test (test for cyclopropanoid, keto and epoxy fatty acid)	Negative
Picric-acid t.l.c test (test for cyclopropanoid, keto and epoxy fatty acid)	Negative
2,4-DNPH t.l.c. test (test for cyclopropanoid, keto and epoxy fatty acid)	Negative
Infrared spectrum for hydroxyl functional group	3460 $\text{cm}^{-1}$
Elemental analysis of Carbon (Cal * 72.48%)	71.23%
Elemental analysis of Hydrogen (Cal * 11.39%)	11.09%
Refractive index at 40°C (highly unsaturated)	1.4730
Reichert-Meissl value (presence of low content steam volatile fatty acids)	0.38
Polenske value of oil (low content of the volatile alcohol soluble and presence of comparatively higher fatty acids in oils)	0.95
Specific rotation (c = 7.0 % in acetone)	$[\alpha]_D^{27} = (+)7.10^\circ$
Specific rotation (c = 6.1 % in chloroform)	$[\alpha]_D^{27} = (+)3.5^\circ$



**FIG 2: STRUCTURE OF 12-HYDROXY-OCTADEC-CIS-9-ENOIC ACID**



**FIGURE 3: MASS FRAGMENTATION PATTERN AND TMSI REARRANGEMENT FRAGMENTATION ION FOR METHYL ESTERS OF 12-HYDROXY-OCTADEC-CIS-9-ENOIC ACID**

Further, the presence of cis double bond at C9 and C10 was confirmed by hydrogenation of unsaturated hydroxy fatty acid with 10% Pd/C in ethanol (5 ml) results in 12-hydroxy stearic acid, m.p. 56-57°C (p-bromophenacyl ester, m.p. 74-75°C). The unsaturated hydroxy acid on oxidation<sup>23</sup> with potassium permanganate in acetic acid resulted products azelaic acid and heptanoic acid.

The m.p. of azealic acid is 106-107°C (p-bromophenacyl ester, m.p. 131-132 °C) and heptanoic acid -7°C (p-bromophenacyl ester, m.p. 66-67°C) and was uncorrected. GLC analysis of the methyl esters of azealic and heptanoic acid showed the cleavage points were between C9 - C10 and C12 - C13.

**In- vitro Antimicrobial screening:****Evaluation of minimal inhibitory concentrations (MIC):**

The MIC value of the ricinoleic acid was carried out using concentrations ranging from 2.5 to 20 mg/ mL. Ricinoleic acid showed significant

inhibition at 2.5 mg/mL against *Escherichia coli*, *Candida albicans* and *Bacillus subtilis*, while compound showed moderate MIC with other test organism when compared *Escherichia coli*, *Candida albicans* and *Bacillus subtilis*. Results of MIC are depicted in **Table 2**.

**TABLE 2: IN VITRO MINIMUM INHIBITION CONCENTRATIONS EVALUATION OF ISOLATED COMPOUND AGAINST STAPHYLOCOCCUS AUREUS, PSEUDOMONAS AUREGINOSA, BACILLUS SUBTILIS, ESCHERICHIA COLI, CANDIDA ALBICANS AND CANDIDA PARAPSILOSIS**

Test Compound	MIC						
	Organism	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>	<i>Candida parapsilosis</i>
Ricinolenic acid		20	*	05	10	10	*

\* indicates values more than 40 mg/mL. The value of each constituents consisted of  $\pm$  S.E.M. of 03 replicates.

**Antimicrobial screening:** The test compound bearing the hydroxyl group and unsaturation in the moiety showed significant inhibiting growth with  $19.01 \pm 0.33$  mm and  $16.17 \pm 0.95$  mm zone of inhibition against *Escherichia coli* and *Pseudomonas aeruginosa* whereas compound showed moderate activity  $11.1 \pm 1.2$  and  $12.33 \pm 0.56$  *Bacillus subtilis* and *Staphylococcus aureus* when compared to the standard drug Ampicillin. Test compound showed moderate activity  $15.33 \pm 1.2$  and  $15.33 \pm 1.2$  zone of inhibition against *Candida albicans* and

*Candida parapsilosis* which produced  $19.3 \pm 0.33$  mm  $18.83 \pm 1.13$  mm zone of inhibition; this was comparable to the effect of the standard Fluconazole used. The antimicrobial study of the compound tested showed significant antimicrobial activity specifically with *Escherichia coli* and *Pseudomonas aeruginosa* compared to other test organism, standard drug ampicillin and Fluconazole. Result of *in-vitro* antimicrobial activity is depicted in **Table 3**.

**TABLE 3: ANTIMICROBIAL ACTIVITY OF THE ISOLATED COMPOUND AGAINST STAPHYLOCOCCUS AUREUS, PSEUDOMONAS AUREGINOSA, BACILLUS SUBTILIS, ESCHERICHIA COLI, CANDIDA ALBICANS AND CANDIDA PARAPSILOSIS**

Compounds	Zone of inhibition (mm)					
	<i>Staphylococcus aureus</i>	<i>Bacillus Subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Candida parapsilosis</i>
Ricinolenic acid	$12.33 \pm 0.56$	$11.1 \pm 1.2$	$19.01 \pm 0.33$	$16.17 \pm 0.95$	$15.33 \pm 1.2$	$15.33 \pm 1.2$
Ampicillin	$17 \pm 1.53$	$14.33 \pm 1.45$	$20.67 \pm 0.33$	$19.67 \pm 0.88$	ND	ND
Fluconazole	ND	ND	ND	ND	$19.3 \pm 0.33$	$18.83 \pm 1.13$

The value of each constituents consisted of  $\pm$  S.E.M. of 03 replicates. ND – Not Defined.

**Acute Toxicity and gross behavioural Studies:**

From the preliminary toxicity studies it indicates that the test compound has revealed good safety profile till the uppermost dose (1500 mg/kg). No mortality and behavioural changes of animals was observed even after 24 h for the compound but mortality was seen in the compound at the concentration 1000 and 1500 mg/kg body weight and behavioural changes were also recorded for same concentration. The experimental studies revealed that ricinoleic acid is quite safe up to 1500 mg/kg and no mortality in animals was recorded.

Further, no significant gross behavioural changes were observed in experimental animals at concentration 1000 and 1500 mg/kg, which showed safe up to 1500 mg/kg.

**Anthelmintic activity:** The anthelmintic activity of the ricinoleic acid showed moderate activity  $59 \pm 1.15$  min. for paralysis and  $114.33 \pm 2.85$  min for the death which is comparably moderate with that of standard Piperazine citrate drug used. The results of the anthelmintic activity comparably moderate activity which is depicted in the **Table 4**.

**TABLE 4: ANTHELMINTIC ACTIVITY OF ISOLATED COMPOUND AGAINST PHERETIMA POSTHUMA**

Test samples	Concentration (mg/ ml)	Time taken for paralysis (min)	Time taken for death (min)
Control	(Normal Saline)	$64.33 \pm 0.88$	$200.33 \pm 2.6$
Ricinolenic acid	50 mg	$59 \pm 1.15$ *	$114.33 \pm 2.85$ **
Piperazine citrate	50 mg	$19.33 \pm 2.6$ **	$40.67 \pm 0.88$ **

The value of each constituents consisted of  $\pm$  S.E.M. of replicates

**CONCLUSION:** The unusual fatty acid isolated from the seed oil of *Swietenia humilis* Zucc. is 12-hydroxy-octadec-cis-9-enoic acid confirmed by the various physicochemical tests, IR, NMR and Mass spectral studies. Further the ricinoleic acid was screened for their biological activity and toxicity studies; the ricinoleic acid showed promising biological activity. Though the plant has significant importance of medicinal and timber aspects, *Swietenia humilis* Zucc. is listed as an endangered species in need of conservation in Appendix II of CITES.

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#### REFERENCES:

1. Dramane S, Mamidou KW and Kamanzi K: Evaluation of antimicrobial and free radical scavenging activities of some bioactive taxons from cote d'ivoire: Eur J Sci Res 2010;40,2: 307-317.
2. Cooke T: The Flora of the Presidency of Bombay. Botanical Survey of India. Calcutta, Vol 1, 1967: 214.
3. Schmidt Lars, Jøke Dorthe. danida forest seed centre. Seed Leaflet No. September 2000.
4. Orwa et al. Agroforestry Database 4.0: 2009.
5. Miguel Ángel Angulo-Escalante, Evangelina Armenta-Reyes, Raymundo Saúl García-Estrada, José Armando Carrillo-Fasio, Edith Salazar-Villa and José Benigno Valdéz- Torres: Extracts of *Swietenia humilis* Zucc. Seed with Antifungal Activity in *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. Revista Mexicana de FITOPATOLOGÍA 2009; 27, 2:85- 92.
6. Veronica Perez-Rubio, Jose Basilio Heredia, Cristobal Chaidez-Quiroz, Jose Benigno Valdez-Torres, Edith Salazar-Villa, Raul Allende-Molar and et al: Physicochemical characterization and fatty acid content of 'venadillo' (*Swietenia humilis* Zucc.) seed oil. African J Biotechnology 2012; 11, 22:6138-42.
7. Jörgen Sjögren, Jesper Magnusson, Anders Broberg, Johan Schnürer and Lennart Kenne: Antifungal 3- Hydroxy Fatty Acids from *Lactobacillus plantarum* MiLAB 14. Appl Environ Microbiol 2003; 69: 12: 7554-57.
8. Hosamani KM and Katagi KS: Characterization and structure elucidation of 12- hydroxyoctadec-cis-9-enoic acid in *Jatropha gossypifolia* and *Hevea brasiliensis* seed oils: a rich source of hydroxy fatty acid. Chemistry and Physics of Lipids 2008; 152: 1: 9-12.
9. Griffin RC, AMSM. Technical Methods of Analysis. P. McGraw-Hill zBook Co. Inc. New York, USA. 1927: 298 & 320.
10. Sharma BK. Industrial Chemistry Goel Publishing House, Meerut, India, 7th Ed., 1955: 506-9.
11. K.M. Hosamani. Terminalia chebula seed oil: A minor source of 12- hydroxy- octadec-cis-9- enoic acid: Natural products as a source for the food and agricultural industries J Sci. Fd. Agric 1994; 64: 275-77.
12. Davis EN, Wallen LL, Goodwin JC, Rohwedder WK and Rhodes RA: Microbial hydration of cis-alkenoic acids. Lipids 1969; 4:356-62.
13. Fioriti JA and Sims RJ: A spray reagent for the identification of epoxides on thin layer plates. J Chromatography 1968; 32: 761-63.
14. W.E. Link, (Ed.). Official and Tentative Methods of the American Oil Chemists Society, third ed. (AOCS, Champaign, IL. 1973).
15. Official and Tentative Methods of the American Oil Chemists Society, AOCS Methods, Champaign, IL, USA, 3rd ed. 1997:15-48 and 1955: DA 148
16. Bharucha KE and Gunstone FD: A New method of determining the Component acids of oils containing epoxy and / or hydroxy acids. Vegetable oils. J Sc Fd Agric 1955; 6:373-80.
17. Shridhar AH, Keshavayy J, Peethambar SK and JoyHoskeriH: Synthesis and biological activities of Bis alkyl 1, 3, 4-oxadiazole incorporated azo dye derivatives. Arabian J of Chemistry 2012 online.
18. Islam MA, Alam MM and Choudhury ME: Determination of minimum inhibitory concentration (MIC) of cloxacillin for selected isolates of methicillin-resistant *Staphylococcus aureus* (mrsa) with their antibiogram. Bangl J. Vet. Med 2008; 6(1):121-26.
19. Lehrer RI, Rosenman M and Harwing SSL: Ultra-sensitive assays for antimicrobial polypeptides. J. Immunol Methods 1991; 137:67-73.
20. Dash GK, Suresh P, Kar DM, Ganpaty S and Panda SB: Evaluation of *Evolvulu salsinoids* Linn. for anthelmintic and antimicrobial activities. J Nat Rem. 2002; 2:182.
21. Tambe VD, Nirmal SA, Jadhav RS, Ghogare PB, Bhalke RD, Girme AS and Bhamber RS: Anthelmintic activity of *Wedelia trilobata* leaves. Ind J Nat Prod 2006; 22:27-29.
22. Kleiman R and Spencer GF: Gas Chromatographic-mass spectrometry of methyl esters of unsaturated oxygenated fatty acids. J Amer Oil Chem Soc. 1973; 50: 31.
23. Bharucha KE, Gunstone FD: Vegetable oils Part-VI. The Component Acids of Ergot oil. J Chem Soc 1957; Part-I, 610.

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