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# *In vitro* Antimicrobial Activity, Lipid Content and Physico-Chemical Analysis of Non-Edible Oils

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Antimicrobial activity of 7 non-edible seed oils on 7 species of microbial organisms were examined against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella sonnei*, *Klebsiella pneumoniae*, *Streptococcus fecalis* and *Shigella dysentrae*. These are examined using agar well diffusion method against 7 bacteria. The seed material of non-edible oil resources were extracted using pet ether by soxhlet apparatus. The extracts were studied for fatty acid profile by gas chromatography and showed presence of palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. Physico-chemical properties like, oil colour, oil content (%), moisture (%), ash (%), specific gravity, refractive index, acid value, iodine value, free fatty acid, saponification value, mean molecular mass, content of nitrogen (%) and protein (%) were carried out. This paper reports on preliminary screening of the *in vitro* antimicrobial activities against 7 human pathogenic bacteria, lipid content and physicochemical analysis of non edible oil yielding plant species.

**Key Words: Seed oil, *In vitro* antimicrobial, Lipid content, Physico-chemical analysis.**

## INTRODUCTION

Plants and plant parts are used for healing purpose is an ancient practice all over the world. World is facing a major problem of health care with infectious diseases which are the leading cause of premature deaths, killing almost 50,000 people every day. It is estimated that there are 250,000-500,000 species of plants on Earth[1].

India is richest country in the world for medicinally important plant resources. Medicinal plants and their products are used to control diverse disease such as catarrh, bronchitis, pneumonia, ulcers and diarrhoea. Hundreds of plant species have been tested for antimicrobial properties. The vast majority has not yet been adequately evaluated[2]. Clinical microbiologists were interested in the topic of antimicrobial plant extracts, because it is very likely that phyto-chemicals will find the way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that, on an average, two or three antibiotics derived from microorganisms are launched each year[3]. According to

National Health Experts, 1,500-2,000 drugs are extracted from various plants[4]. Antimicrobial activity of various seed oils *viz*. *Mimusops hexandra*, *Prunus amygdalus*[5], *Brassica alba*[6], *Cardiospermum halicacabum*[7], *Aegle marmelos*, *Anacardium occidentale*[8], *Jatropha gossypifolia*[9], *Hevea brasiliensis*, *Psoralea corylifolia*[10] and *Bauhinia retusa* has reported[11]. The present paper deals with the screening of non-edible oils for antimicrobial activity. The pathogenic organisms of bacteria were selected for the investigations on the basis of their clinical and pharmaceutical importance.

Tribal or local people are using regionally suitable plant resources having non-edible oil for medicinal value. The species which are evaluated in present work for antimicrobial and chemical analysis includes like-*Bauhinia roxburghiana* Voigt (BR), *Caesalpinia crista* L. (CC), *Celastrus paniculata* Willd (CP), *Calophyllum inophyllum* L. (CI), *Madhuca longifolia* (Koen.) Mac Bride (ML), *Sterculia guttata* Roxb. (SG)) and *Scleichera oleosa* (Lour.) Oken (SO).

## EXPERIMENTAL

**Seed samples:** Well dried and matured fruits were collected from Maharsahtra and Karnataka state, India during appropriate season. Seeds were dried under shade for few days and used for further analysis.

**Extraction of oil from seeds:** The seeds were crushed in a grinder machine at 37 °C for few minutes. Oil was extracted with petroleum ether (60-80 ºC) in a Soxhlet apparatus for 6 h. Solvent was removed under reduced temperature and pressure. The yield of oil was calculated using single sample with three replicates.

**Microbial cultures and growth conditions:** Microbial cultures used were obtained from Microbial Sciences Division of Agharkar Research Institute (ARI), Pune and National Chemical Laboratory, Pune which includes-*Bacillus cereus* (+ ve) MCMB-817, *Staphylococcus aureus* (+ve) MCMB-818, *Escherichia coli* (-ve) MCMB-813, *Shigella sonnei* (-ve) MCMB-815, *Klebsiella pneumoniae* (-ve) NCIM-5082, *Streptococcus fecalis* (-ve) NCIM-5024 and *Shigella dysentrae* (-ve). Microbial cultures were grown on Mueller-Hinton agar at 37 °C for 12-14 h. They were maintained at 4 °C in the laboratory. Standard antibiotic-Octo discs (O.D. 019), (HiMedia).

**Antimicrobial assay:** Antibacterial activity of seed oil was carried out by modified agar well diffusion method[12,13]. The test organisms maintained on agar slants were recovered for testing by inoculating into nutrient broth and incubated at 37 ºC in a shaker at 180 rpm. Eighteen hours culture of each microorganism was diluted in sterile saline to an absorbance of approximately 0.11 O.D. ($10^5$ CFU) on UV spectrophotometer at 620 nm. 0.1 mL of above culture was inoculated in plates containing MH agar and spread evenly using sterile glass spreader. These plates were incubated at 37 ºC for 0.5 h. All seed oils were tested at two different concentrations, *viz*. 300 and 500 mcg in MH medium. Test extracts were incorporated into the wells made by sterile 5 mm size borer in media and different concentrations of extracts were added along with control dimethyl sulphoxide in wells and octo-discs of gentamycin

10 µg and tetracycline 100 µg was used as positive control. Plates were incubated and observed after 24 h. All the experiments were done in triplicate. The diameters of zones of inhibition (mm) are expressed as means and standard errors on means.

**Gas chromatography:** Seed powders were used for determination of FA[14]. The FA was estimated on Agilent 6890 N gas chromatograph with auto sampler and auto injector. The samples were injected in 30 mm long, 0.32 mm diameter HP-Innovax capillary column. Auto injector, oven temperature and flame ionization detector were adjusted to 225, 115 and 275 °C, respectively. The initial oven temperature was 150 °C ramped by 15 °C/min up to 250 °C/min. Flame ionization detector (FID) was used to detect the signals. Hydrogen and air with flow rates of 30 and 400 mL/min, respectively, were used to ignite the flame of FID. Nitrogen gas (2 mL/min) was used as carrier gas. Standard fatty acids of Sigma Chemicals Ltd. were used as standard to calibrate the method. The signals from the detector were integrated as normal percentages of calibration curve by using HP chemstation software.

**Physico-chemical properties:** The iodine value (IV), saponification value (SV) and acid value (AV) of the oil were determined by standard procedures described in the literature[15,16]. The mean molecular mass (MMM) was estimated from the equation (560/SV) × 100[9-11,17-19]. The free fatty acid (FFA) was calculated from the relationship given *i.e.*, 1 unit of acid value ≠ 0.503 % FFA (calculated as oleic acid)[16]. The specific gravity was determined by the method[20]. Refractive index was carried out by refractometer at 20 ºC (Erma Tokyo No. 6343) Table-1. Crude nitrogen was determined by Kjeldhals method and protein was calculated by NX 6.25[21]. Ash content was carried out in muffle furnace at 560 ºC and moisture content by sartorius MA 45 autoanalyser at 100 ºC.

## RESULTS AND DISCUSSION

The major components of all the tested seeds were palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. Compositions of the fatty acid (FA) esters are given in Table-2. Microorganisms convert oleic and linoleic acids to many oxygenated fatty acids[22]. Many ω-3 and ω-6 PUFAs are converted to their corresponding oxygenated PUFAs[23,24]. All of these new products have high potential for antimicrobial agents or biomedical applications[25]. It is assumed that oxidative effect could plausibly play an important role in the antimicrobial function of fatty acids[26].

Antibacterial activity of the different plant oils was presented in Table-1. Total 7 strains of different disease causing organism were tested against 7 seed oils. The bacterial strains like *S. aureus* and *K. pneumoniae* demonstrated more sensitivity to the oils tested followed by *B. cereus* with zone of clearance ranging 3-8 mm. The *E. coli* and *S. dysentrae* showed moderate activity. While *S. sonnei* and *S. fecalis* showed more resistance to the tested oils. The standard antibiotics gentamycin and tetracycline showed zone diameter ranging 5.0-16.0 mm. The zones of inhibitions of oils are found within the range of standard antibiotics.

TABLE-1
SCREENING RESULTS FOR ANTIMICROBIAL ACTIVITY OF THE OIL SEEDS AND STANDARD ANTIBIOTICS BY AGAR WELL DIFFUSION METHOD

| *Bauhinia roxburghiana* Voigt | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name of micro-organism (zone of inhibition in mm) | | | | | | | |
| Conc. (µg) | Bc | Sa | Ec | Ss | Kp | Sd | Sf |
| 300 | NI | NI | 3.0 | NI | 3.5 | NI | NI |
| 500 | NI | NI | 4.0 | NI | 4.0 | NI | NI |
| *Caesalpinia crista* L. | | | | | | | |
| 300 | 6.0 | NI | NI | NI | NI | NI | 4.5 |
| 500 | 6.5 | NI | NI | NI | 3.5 | NI | 5.0 |
| *Calophyllum inophyllum* L. | | | | | | | |
| 300 | 5.5 | 7.5 | NI | NI | NI | NI | NI |
| 500 | 6.0 | 8.0 | NI | NI | NI | NI | NI |
| *Celastrus paniculata* Willd | | | | | | | |
| 300 | NI | 4.5 | 3.5 | NI | 3.0 | NI | NI |
| 500 | NI | 5.0 | 4.0 | 4.0 | 3.5 | 4.0 | NI |
| *Scleichera oleosa* (Lour.) Oken | | | | | | | |
| 300 | 6.0 | 4.0 | 6.0 | NI | 4.0 | 4.5 | 5.0 |
| 500 | 6.5 | 4.5 | 7.0 | NI | 4.5 | 5.0 | 6.0 |
| *Madhuca longifolia* (Koen.) Mac Bride | | | | | | | |
| 300 | NI | NI | NI | NI | 3.0 | 3.0 | NI |
| 500 | NI | 4.0 | NI | NI | 3.5 | 5.0 | NI |
| *Sterculia guttata* Roxb. | | | | | | | |
| 300 | 5.5 | NI | NI | NI | NI | NI | NI |
| 500 | 6.0 | 5.0 | NI | NI | NI | NI | NI |
| Gentamycin (GM) and Tetracyclin (TC) | | | | | | | |
| 10 (GM) | 16 | 15 | 11 | 16 | 15 | 12 | 16 |
| 100 (TC) | 16 | 16 | 14 | 8.0 | NI | 5.0 | 14 |

NI: No Inhibition. Bc = *Bacillus cereus*, Sa = *Staphylococcus aureus*, Ec = *Escherichia coli*, Ss = *Shigella sonnei*, Kp = *Klebsiella pneumoniae*, Sd = *Shigella dysentrae*, Sf = *Streptococcus fecalis*.

The fatty acid profile present in the mixture is identified by comparison against the standard fatty acid esters. Saturated fatty acid (SFA) and unsaturated fatty acid (UFA) content in all seeds are-*Bauhinia roxburghiana* Voigt-289.1 and 710.8 mg/g, *Caesalpinia crista* L.-196.6 and 803.3 mg/g, *Calophyllum inophyllum* L.-307.1 and 703.1 mg/g, *Celastrus paniculata* Willd-259.4 and 740.6 mg/g, *Madhuca longifolia* (Koen.) Mac Bride-440.5 and 559.4 mg/g, *Sterculia guttata* Roxb.-385.2 and 614.8 mg/g and *Scleichera oleosa* (Lour.) Oken-197.4 and 802.5 mg/g. FA determined by GC showed that these are mostly unsaturated oils[27].

The physico-chemical properties are given in Table-3. Seed oil obtained from *Bauhinia roxburghiana* Voigt and *Caesalpinia crista* L. is yellowish brown, *Celastrus paniculata* Willd and *Scleichera oleosa* (Lour.) Oken yellow, *Calophyllum inophyllum* L. greenish, *Madhuca longifolia* (Koen.) Mac Bride whitish yellow and *Sterculia guttata* Roxb. brown in colour and remains in non-drying state at

room temperature[28,29]. The content of oil in *Calophyllum inophyllum* L. is high, ranging up to 70.64 % and least in *Sterculia guttata* Roxb. *i.e.,* 24.0 % by weight of the seeds, respectively. This value is close to those obtained from the other common oil-bearing seeds. Moisture content is in range 3.1-7.1 % which lies within the range of other non-edible oil seeds. Ash values are 0.96-0.98 %, respectively.

TABLE-2
FATTY ACID COMPOSITION OF SEED OILS (%)

| Fatty acid | Formula | Structure | BR | CC | CI | CP | ML | SG | SO |
|---|---|---|---|---|---|---|---|---|---|
| Palmitic acid | $C_{16}H_{32}O_2$ | 16:0 | 18.58 | 14.35 | 17.20 | 22.98 | 25.81 | 22.07 | 13.66 |
| Stearic acid | $C_{18}H_{36}O_2$ | 18:0 | 10.33 | 5.31 | 13.51 | 2.96 | 18.24 | 16.45 | 6.08 |
| Oleic acid | $C_{18}H_{34}O_2$ | 18:1 | 26.11 | 38.57 | 34.90 | 32.54 | 39.92 | 40.25 | 70.36 |
| Linoleic acid | $C_{18}H_{32}O_2$ | 18:2 | 44.97 | 41.76 | 25.35 | 15.59 | 16.02 | 21.23 | 9.89 |
| Linolenic | $C_{18}H_{30}O_2$ | 18:3 | – | – | 10.06 | 25.93 | – | – | – |

BR: *Bauhinia roxburghiana* Voigt, CC: *Caesalpinia crista* L., CI: *Calophyllum inophyllum* L., CP: *Celastrus paniculata* Willd, ML: *Madhuca longifolia* (Koen.) Mac Bride, SG: *Sterculia guttata* Roxb., SO: *Scleichera oleosa* (Lour.) Oken.

TABLE-3
PHYSICO-CHEMICAL PROPERTIES OF SEED OILS

| Properties | BR | CC | CI | CP | ML | SG | SO |
|---|---|---|---|---|---|---|---|
| Oil colour | Yellowish brown | Yellowish brown | Greenish | Yellow | Whitish yellow | Brown | Yellow |
| Oil (fat %) | 30.39 | 32 | 70.64 | 52 | 48 | 24 | 41 |
| Moisture (%) | 5.01 | 3.1 | 5.8 | 6.4 | 7.1 | 6.5 | 5.5 |
| Ash (%) | 0.96 | 0.97 | 0.98 | 0.97 | 0.97 | 0.97 | 0.96 |
| SG at RT (28 °C) | 0.913 | 0.952 | 0.943 | 0.959 | 0.924 | 0.904 | 0.908 |
| RI (20 ºC) | 1.48 | 1.48 | 1.47 | 1.47 | 1.47 | 1.46 | 1.47 |
| Acid value (mg/g) | 2.72 | 3.88 | 31.25 | 18.53 | 7.64 | 1.40 | 42.20 |
| Iodine value (g/100 g) | 272.9 | 51.04 | 94.87 | 103.4 | 35.58 | 146.4 | 41.4 |
| Saponification value (mg KOH/g) | 321.7 | 347.7 | 364.0 | 239.2 | 550.7 | 574.6 | 564.8 |
| Mean molecular mass | 174.1 | 166.6 | 154.4 | 233.9 | 101.6 | 97.0 | 99.0 |
| Nitrogen (%) | 6.10 | 1.70 | 3.12 | 1.93 | 1.70 | 2.79 | 2.70 |
| Protein (%) | 38.33 | 10.62 | 17.82 | 12.10 | 10.67 | 17.43 | 16.90 |

BR: *Bauhinia roxburghiana* Voigt, CC: *Caesalpinia crista* L., CI: *Calophyllum inophyllum* L., CP: *Celastrus paniculata* Willd, ML: *Madhuca longifolia* (Koen.) Mac Bride, SG: *Sterculia guttata* Roxb., SO: *Scleichera oleosa* (Lour.) Oken.

Specific gravity of all seed oil is determined by specific gravity bottle and is ranging 0.904-0.959 at 28 °C. These values are comparable with most of the seed oils. Refractive index is determined by Erma Tokyo No. 6343 refractometer at 20 ºC and it is found in range1.46-1.48 in all. These values are slightly higher than the most of the seed oils.

The acid value is found in range 1.40-42.20 mg KOH/g in all oils. These values are within the range of reported oils. Acid value up to 6.2 mg $KOH/g^{-1}$ is suitable for *trans*-esterification[26]. Iodine value is found to be in the range 41.4-272.9 g/100 g, respectively. Iodine value in most of the vegetable oils was observed within the range 104-132 g/100 g[30].

Saponification value is found to be 239.2-564.8 mg KOH/g, respectively. These values are high as compared to other vegetable oils. Saponification values of common vegetable oils are found to be approximately 190-196 mg KOH/g. Mean molecular mass of *Bauhinia roxburghiana* Voigt and *Aphanamixis polystachya* (Wall.) parker[31] oil is found to be 174.1 and 159.51, respectively.

Nitrogen content of seed powder is in the range of 1.7-6.1 %, respectively. These values are comparable with other oils. Protein content is in the range 10.62-38.33 %, respectively. These values lie within the range of other vegetable oils[32].

This study is a preliminary evaluation of antimicrobial activity of the non-edible oils. It indicates that several plants have the potential to generate novel metabolites. Plants demonstrating broad spectra of activity to discover new chemical classes of antibiotics and provide biochemical tools for the study of infectious diseases have been proved.

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