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## Phytochemical Screening and Antioxidant Potential of *Thespesia populnea* (L.) Seed Extract

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### ABSTRACT

Herbal medicines based on the plant *Thespesia populnea* (L.) used in the folk medicine of different cultures of India and some parts of world. In the present research, an attempt was made to extract some medicinally important compounds from the seeds of *Thespesia populnea* (L.) by soxhlet extraction n-butanol as a solvent. The investigation was carried out to determine the chemical components of *Thespesia populnea* (L.) seeds using Perkin-Elmer Gas Chromatography–Mass Spectrometry (GC-MS), while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC-MS analysis revealed the presence of six compounds. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. The results of the study suggest that the n-butanol extract possesses anti-oxidant activity.

**Keywords:** *Thespesia populnea* Seed Extract, n-butanol, GC-MS Analysis, Antioxidant activity.

### INTRODUCTION

Nature is used as source of medicinal agents since times immemorial. Plants are source of medicine has been an ancient practice and becomes an important component of health care system in India. India is leading producer of medicinal herbs so it is called as botanical garden of the World. Plants have played a crucial role in maintaining human health and improving the quality of human life for thousands of years. The World Health Organization has estimated that 80 % of the earth's inhabitants rely on traditional medicine for their health care needs [1], and most of this therapy involves the use of plants extracts or their active components [2]. The global thrust areas for drugs from medicinal plants include disease conditions, whose incidence is unavailable or unsatisfactory. International market of medicinal plants is over US \$ 60 billion per year, which is growing at the rate of 7 % annually [3]. In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species.

*Thespesia populnea* Linn commonly called as Hibiscus Populnea belongs to the family: Malvaceae. *T. populnea* is an evergreen tree. *Thespesia populnea* is a shrub or medium-sized evergreen tree, up to 20 m tall with a dense crown. Bark grayish. Twigs densely covered with brown to silvery scales, glabrescent. Fruit a globose capsule (Figure 1) [4]. Different parts of this plant such as bark, root, fruit and leaf are also used in psoriasis, scabies, hemorrhoids, chronic dysentery, cutaneous and anti-inflammatory diseases [5]. The main medicinal uses are cutaneous infections, skin and liver diseases. Fruit juices are used on rheumatism sprains, scabies, swellings, insect bites and warts. Pulp of fresh fruits are applied for relief of migraine. Unripe fruit juice is used to cure piles. Decoction of bark is given to treat diarrhea and arthritis [6]. The various chemical constituents were isolated from the *T. populnea* are Gossypol [7], 7 Hydroxy-2,3,5,6-tetrahydro-3,6,9-Trimethylnaphto [1,8-B,C] Pyran-4,8-Dione, Kaempferol, Quercetin, Kaempferol 3-glucoside, Quercetin 3-glucoside, rutin, Nonacosane, lupenone, myricyl alcohol, lupeol,  $\beta$ -sitosterol and  $\beta$ -sitosterol- $\beta$ -D-glucoside, 5, 8-dihydroxy-7-methoxyflavone, 7-hydroxyisoflavone and Thespones, Mansonones D E and F Populneol, Thespesin [8]. Metal oxides play a very important role in many

areas of chemistry, physics and materials science. The metal elements are able to form a large diversity of oxide compounds. In technological applications, oxides are used in the fabrication of microelectronic circuits, sensors, piezoelectric devices and fuel cells, coatings for the passivation of surfaces against corrosion and as catalysts [9-32]. Rao et al. have reported their work on different oxide materials in their earlier studies [33-63]. In line with this, the present study was designed to explore the chemical constituents, and antioxidant effect of *T. populnea* seed extract using established in vitro method. This work will help to identify the compounds, which may be used in body products or of therapeutic value.



Figure 1: *Thespesia populnea* (L) Leaves and seeds (Source: [www.indiabiodiversity.org](http://www.indiabiodiversity.org))

## MATERIALS AND METHODS

**Chemicals:** All Chemicals used in the entire study were AR grade obtained from SD fine chemicals, India, Pvt Ltd.,

**Plant Material:** Dry seeds of *Thespesia populnea* (L) collected (in the month of April 2015) from surrounding areas of rural areas of Vijayawada, Krishna District. The plant material was authenticated at the Botanical Survey of India, Howrah and West Bengal, India.

**Preparation of plant extract:** The dry material of *T. populnea* (L) passed through sieve (100  $\mu$ ). The coarse powdered drug (125 gm) was extracted in Soxhlet apparatus for 28 h with n-butanol (40-50 °C, 2 L). n-butanol extract obtained was concentrated under reduced pressure in rotatory evaporator below 55 °C temperature to get semisolid sticky residue (15 gm).

**Column chromatography:** n-butanol extract of the plant material (5 g) was subjected to column chromatography using silica gel (80 - 120 #) as adsorbent and eluted with the mixture of n-butanol: ethyl acetate in gradient manner. n-butanol: ethyl acetate (60: 40) fraction yielded dark brown color liquid.

**GC-MS Analysis:** GC-MS analysis of the extract was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30 mm x 0.25 mm ID x 1  $\mu$ Mdf, composed of 100 % Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999 %) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2  $\mu$ l was employed (split ratio of 10:1); Injector temperature 250 °C; Ion-source temperature 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes.

**Identification of components:** Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2. This is done in order to determine whether this plant species contains any individual compound or group of

compounds, which may substantiate its current commercial and traditional use as an herbal medicine. Further it helps to determine the most appropriate methods of extracting these compounds. These results will consequently be discussed in the light of their putative biological or therapeutic relevance. The name, molecular weight and structure of the components of the test materials were ascertained (Table 1 & Figure 2).

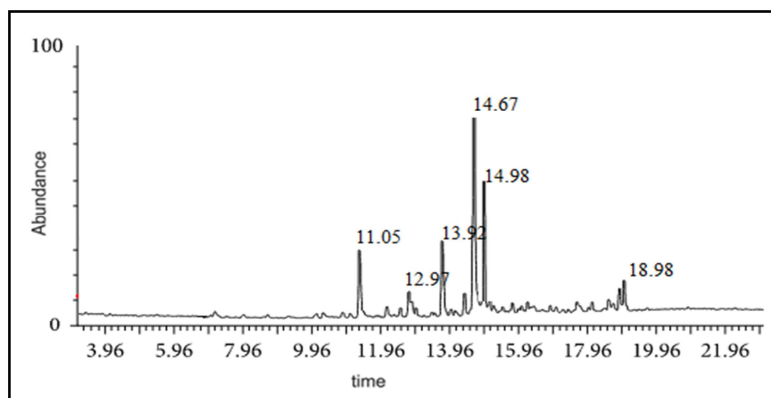


Figure 2: GC-MS chromatogram of n-butanol extract of *T. populnea* (L)

Table 1: Components detected in n-hexane seed extract of *T. populnea* (L.) and its biological activities obtained through the GC-MS study

S.No.	RT	Name of the compound	Molecular formula	MW	Peak Area %	Nature of compound	Activity#
1	11.05	ethyl 4-(hexadecahydro-3,7,12-trihydroxy-10,13-dimethyl-1H-cyclopenta [a] phenanthren-17-yl) pentanoate (Ethyl iso-allocholate)	C <sub>26</sub> H <sub>44</sub> O <sub>5</sub>	436	14.54	Steroid	Antimicrobial, anticancer antiarthritic, antiasthma, diuretic, anti-inflammatory
2	12.97	Ethyl palmitate (Hexadecanoic acid, ethyl ester)	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	4.7	Palmitic acid ester	Antioxidant, hypocholesterolemic nematocide, pesticide, anti androgenic, flavor, hemolytic, 5-alpha reductase inhibitor
3	13.92	Linoleic acid ethyl ester	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308	14.97	Linoleic acid ethyl ester	Hypocholesterolemic, nematocide, antiarthritic, hepatoprotective, anti androgenic, hypocholesterolemic 5-alpha reductase inhibitor, antihistaminic, anticoronary, insectifuge, antieczemic, antiacne
4	14.67	Stigma Sterol	C <sub>29</sub> H <sub>48</sub> O	412	47.82	Steroid	Antimicrobial, anticancer, antiarthritic, antiasthma, diuretic, anti-inflammatory
5	14.98	(E)-3,7,11,15-tetramethylhexadec-2-en-1-ol	C <sub>20</sub> H <sub>40</sub> O	296	15.67	Terpene alcohol	Antimicrobial, anti-inflammatory
6	18.98	(6E,10E,12E,16Z)-2,6,10,13,17,21-hexamethyl docosa-2,6,10,12,16,20-hexaene (Squalene)	C <sub>30</sub> H <sub>50</sub>	410	2.3	Triterpene	Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor, pesticide

# Source: Dr. Duke's : Phytochemical and Ethnobotanical databases

Table 2: Display the presence/ absence of different Phytochemicals in the seeds of *T. populnea* (L)

Alkaloids	Dragendroff's test/Mayer's test	+
Terpenoids	2ml CHCl <sub>3</sub> + 2ml Con.H <sub>2</sub> SO <sub>4</sub>	+
Flavonoids	Shinoda test/Lead acetate tests	-
Tannins	Gelatin test/Ferric chloride test	+
Steroids	Salkowski tests/Lieberman Burchardt tests	+
Saponins	Foam test/Froth test	+
Phenols	FeCl <sub>3</sub> test/Liebermann's test	-
Coumarines	Alcoholic sodium hydroxide	-
Anthraquinone	Borntragers test	-

**Phytochemical Screening:**

**Qualitative profiling:** n-Hexane extract of Croton was used for qualitative assessment for the major classes of phytochemicals namely steroids, tannin, acids, esters, terpenoids, alkaloid, carbohydrate and protein etc. The tests were performed according to various standard methods [64]. The tests were based on the visual observation of color change or formation of a precipitate after the addition of specific reagents as shown in Table 2.

**In vitro antioxidant assays:** The in vitro antioxidant assays were carried out by preparing the plant samples (1 mg/ml) in 95 % methanol and then making its serial dilutions. The specific protocol was followed for finding specific scavenging activities of the plant samples.

**Hydroxyl radical scavenging assay:** The power of scavenging hydroxyl free radicals refers to the antioxidant potential of plant sample using methodology practiced by [65]. This technique involved the mixing of 500 µl of 2-deoxyribose (2.8 mM) prepared in 50 mM phosphate buffer and its pH was maintained at 7.4. The reaction mixture was prepared by addition of 100 µl of 0.1 M EDTA, 200 µl of ferric chloride (100 mM) and 100 µl of 200 mM H<sub>2</sub>O<sub>2</sub> and 100 µl of plant sample. The initiation of reaction was brought by the introduction of 100 µl of ascorbic acid (300 mM) and incubated for 1 h at 37 °C. Then 1 ml of 2.8 % trichloroacetic acid and 1 ml of 1 % w/v thiobarbituric acid prepared in 50 mM NaOH were added to the reaction mixture. The whole recipe was heated in water bath for 15 min. After cooling to room temperature the absorbance of the reaction mixture was recorded at 532 nm. The hydroxyl radical scavenging activity was analyzed by the following formula:

$$\text{Scavenging effect \%} = [1 - \frac{\text{Absorbance of the sample}}{\text{Absorbance of control}}] \times 100$$

**RESULTS AND DISCUSSION**

After the successful conventional hot soxhlet extraction of the seed extract in investigation, the preliminary phytochemical study revealed that n-butanol extract of *Thespesia populnea* (L) contains alkaloids, steroids, terpenoids, and saponins, Cardenolides, Coumarines, Anthraquinone were absent in the extract. The GC-MS spectrum confirmed the presence of six components with different retention times as illustrated in [Figure 2]. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. The present study helps to predict the formula and structure of 6 biomolecules. Further investigation may lead to isolation of bio-active compounds and their structural elucidation and screening of pharmacological activity will be helpful for further drug development.

Preliminary phytochemical screening of the leaf extract of *T. populnea* revealed the presence of bioactive constituents such as ethyl 4-(hexadecahydro-3,7,12-trihydroxy-10,13-dimethyl-1H-cyclopenta [α] phenanthren-17-yl) pentanoate (Ethyl iso-allocholate) (14.54 %), Ethyl palmitate (Hexadecanoic acid, ethyl ester) (4.7 %), Linoelic acid ethyl ester (14.97 %), Stigma Sterol as major component (47.82 %), (E)-3,7,11,15-tetramethylhexadec-2-en-1-ol (15.67) and the minor compound identified as (6E,10E,12E,16Z)-2,6,10,13,17,21-hexamethyldocosa-2,6,10,12,16,20-hexaene (Squalene) (2.3 %). Previous reports suggest that the presence of these bioactive constituents in plant preparations could contribute to the antioxidant, antibacterial, antifungal Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor, pesticide, Antimicrobial, anticancer, antiarthritic, antiasthma, diuretic, anti-inflammatory etc., (Table 1). However, further studies are needed to undertake its bioactivity and toxicity profile. From Figure 3 to Figure 8 show the mass spectrum and structure of isolated bio active molecules from *T. populnea* (L) stem bark n-butanol extract.

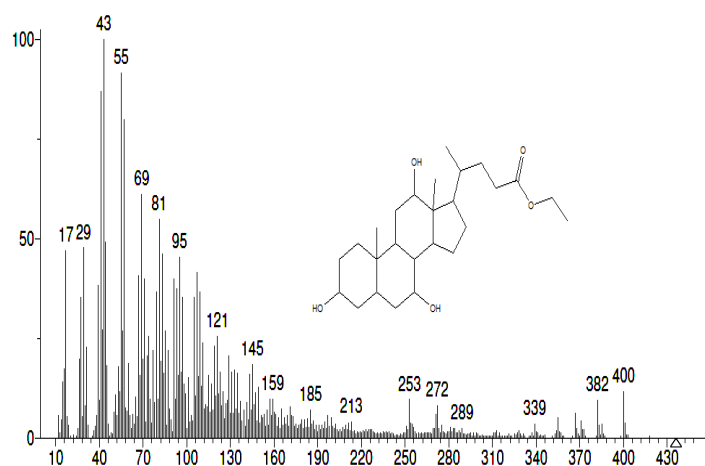


Figure 3: Ethyl 4-(hexadecahydro-3, 7, 12-trihydroxy-10, 13-dimethyl-1H-cyclopenta [α] phenanthren-17-yl) pentanoate (Ethyl isoallocholate)

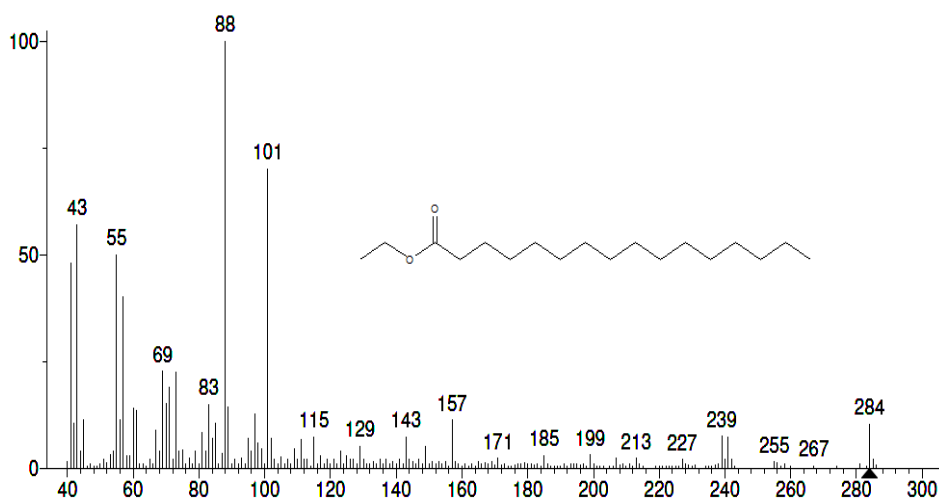


Figure 4: Ethyl palmitate (Hexadecanoic acid, ethyl ester)

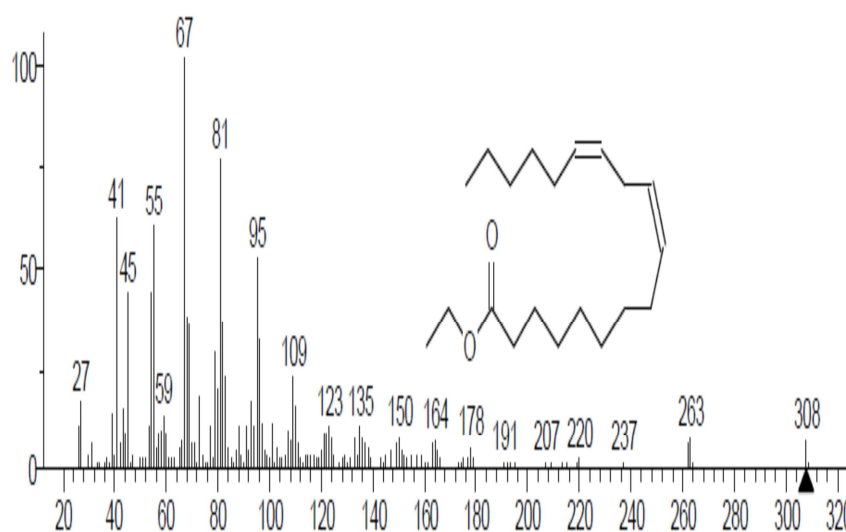


Figure 5: Mass Spectrum and structure of Linoelic acid ethyl ester

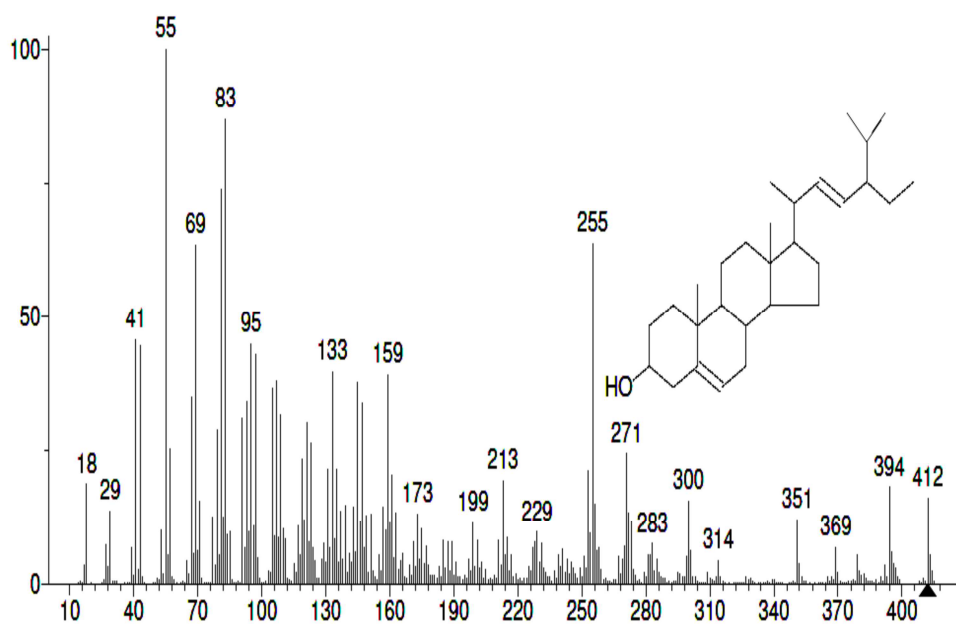


Figure 6: Stigma Sterol

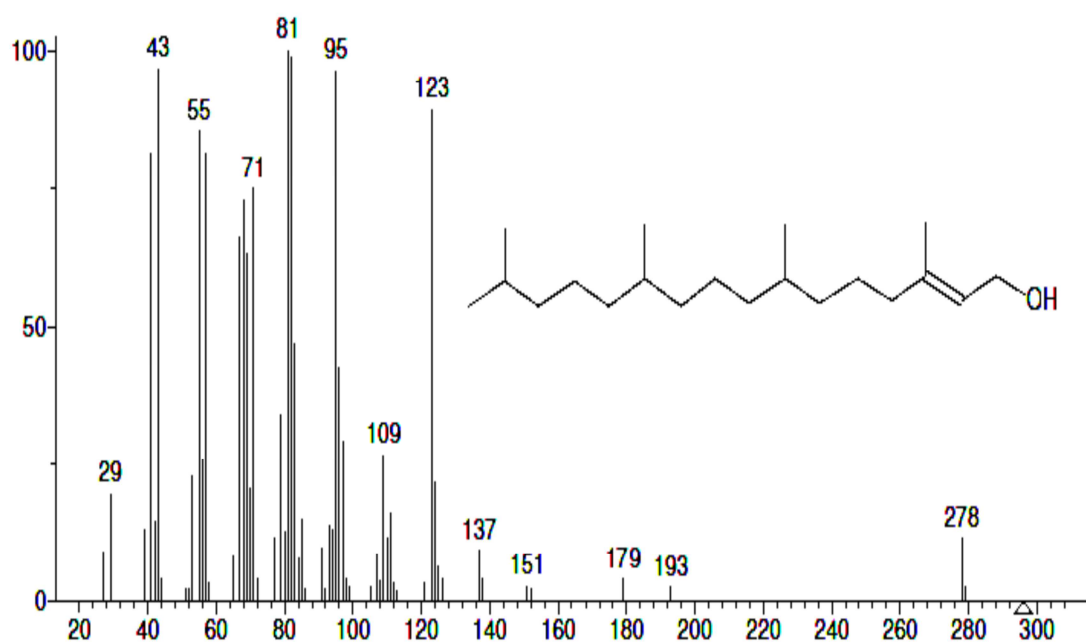


Figure 7: (E)-3, 7, 11, 15-tetramethylhexadec-2-en-1-ol



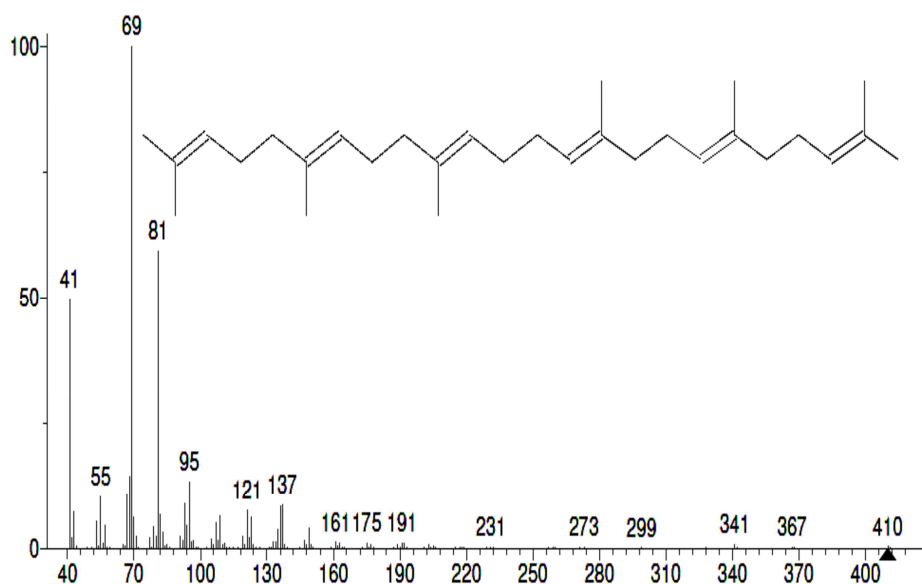


Figure 8: Squalene

**In vitro antioxidant activity:** Hydroxyl radical ( $\bullet\text{OH}$ ) scavenging assay: The seed extract of *T. populnea* (L) scavenged  $\bullet\text{OH}$  radicals and prevented 2-deoxyribose breakdown. A concentration-dependent pattern was observed for hydroxyl radical scavenging activity.  $\text{IC}_{50}$  ( $\mu\text{g/ml}$ ) values of seed extract antioxidant activity was found to be  $254.10 \pm 1.20a$  (Values are presented as means  $\pm$  SD ( $n = 3$ ). Means with different superscript (a) letter in the column is significantly different ( $P < 0.01$ ).

### CONCLUSION

In conclusion, from the results of the present investigation, it could be inferred that *T. populnea* seed is found to have significant medicinal activities. Phytochemical screening and GC-MS study substantiate that *T. populnea* seed extract contain pharmacologically active principles. The phytochemical analysis of the extracts revealed the existence of various constituents including steroids, terpenoids, esters, acids, tannins etc. The active constituent needs to be isolate and should be considered for further in-vivo or in-vitro studies to confirm the tradition. Biological study inferred that, seed extract active as anti oxidant.

### REFERENCES

- [1]B. Sandhya, S. Thomas, N. Isabel, R. Shenbagarathi, *Complementary and Alternative Medicine*, **2006**, 3, 101-114.
- [2]J. Bruneton, C. K. Hatton, Pharmacognosy, translator Paris Lavousier publisher, **1995**, 30-35.
- [3]D. N. Tewari, Report of the taskforce on conservation and sustainable use of medicinal plants. Planning commission, **2000**, 1-18.
- [4]C. Orwa, A. Mutua, R. Kindt, R. Jamnadass, S. Anthony, Agroforestry Database: a Tree reference and selection guide version 4.0, **2009**.
- [5]A. Asima chatterjee, S.C.Pakrashi, The treatise on Indian Medicinal Plants, **2009**, 2:188.
- [6]B. Jean B, Phytochemistry of Medicinal plants, 2nd Edn., New York: Intercept Ltd., **1999**, 225-369.
- [7]A. Mohini, J. Phanse Manohar, Patil Konde Abbulu., *Int. J Pharm. Pharm. Sci.*, **2015**, 5(3), 1-5.
- [8]Y. A. Bekro, J. A. M. Bekro, B. B. Boua, F. H. Tra Bi, E. E. Ehil , *Rev. Sci. Nat.*, **2007**, 4(2), 217-225.
- [9]M.C. Rao, O.M. Hussain, *Res. J. Chem. Sci.*, **2011**, 1, 92-95.
- [10] M.C. Rao, *J. Non-Oxide Glasses*, **2013**, 5, 1-8.
- [11] M.C. Rao, O.M. Hussain, *Res. J. Chem. Sci.*, **2011**, 1 (7), 76-80.
- [12] M.C. Rao, *Res. J. Recent. Sci.*, **2013**, 2(4), 1-8.
- [13] M.C. Rao, *Int. J. Mod. Phys., Conf. Series*, **2013**, 22, 576-582.
- [14] M.C. Rao, *Int. J. Chem. Sci.*, **2012**, 10(2), 1111-1116.
- [15] M.C. Rao, K. Ramachandra Rao, *Int. J. Chem Tech Res.*, **2014**, 6(7), 3931-3934.
- [16] S.M. Begum, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Inorg. Organometa. Poly. Mater*, **2013**, 23(2), 350-356.
- [17] S.M. Begum, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Mol. Struct.*, **2011**, 1006(1), 344- 347.
- [18] S.M. Begum, M.C. Rao, R.V.S.S.N. Ravikumar, *Spectrochim. Acta Part A: Mol. & Biomol. Spec.*, **2012**, 98, 100-104.

- [19] K. Ravindranadh, RVSSN Ravikumar, M.C. Rao, *Int. J. Mod. Phys., Conf. Series*, **2013**, 22, 346-350.
- [20] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Mater. Sci: Mater. Elect.*, **2015**, 26, 6667-6675.
- [21] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *Appl. Mag. Reson.*, **2015**, 46(1), 1-15.
- [22] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Luminesce.*, **2015**, 159, 119-127.
- [23] M.C. Rao, *J. Crys. Growth*, **2010**, 312(19), 2799-2803.
- [24] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2011**, 5, 85-88.
- [25] M.C. Rao, O.M. Hussain, *IOP Conf. Series: Mater. Sci. Eng.*, **2009**, 2, 012037 (p.1-4).
- [26] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2011**, 5(5-6), 651-654.
- [27] M.C. Rao, *J. Optoelect. & Adv. Mater.*, **2011**, 13, 428-431.
- [28] M.C. Rao, O.M. Hussain, *Optoelect. & Adv. Mater.*, **2011**, 13(2-4), 1109-1113.
- [29] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2012**, 6, 511-515.
- [30] M.C. Rao, O.M. Hussain, *Eur. Phys. J. Appl. Phys.*, **2009**, 48(2), 20503 (p.1-6).
- [31] M.C. Rao, O. M. Hussain, *Ind. J. Eng. Mater. Sci.*, **2009**, 16, 335-340.
- [32] M.C. Rao, *J. Optoelect. & Adv. Mater.*, **2010**, 12, 2433-2436.
- [33] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2010**, 4, 2088-2091.
- [34] M.C. Rao, *J. Optoelect. & Adv. Mater.*, **2011**, 13, 78-81.
- [35] M.C. Rao, K. Ravindranadh, Sk. Muntaz Begum, G. Nirmala, *AIP Conf. Proc.*, **2011**, 1349, 641-642.
- [36] M.C. Rao, O. M. Hussain, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2012**, 6, 263- 266.
- [37] M.C. Rao, *Res. J. Chem. Sci.*, **2012**, 2(3), 74-79.
- [38] M.C. Rao, Sk. Muntaz Begum, E.Sivanagi Reddy, O.M.Hussain , *AIP Conf. Proc.*, **2012**, 1447, 613-614.
- [39] M.C. Rao, S.M. Begum, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2012**, 6, 508-510.
- [40] M.C. Rao, O.M. Hussain, *J. Alloys Compd.*, **2010**, 491(1), 503-506.
- [41] M.C. Rao, K. Ravindranadh, *Der Pharma Chemica*, **2016**, 8, 243-250.
- [42] M.C. Rao, K. Ravindranadh, M.S. Shekhawat, *AIP Conf. Proc.*, **2013**, 1536, 215-216.
- [43] K. Ravindranadh, M.S. Shekhawat, M.C. Rao, *AIP Conf. Proc.*, **2013**, 1536, 219-220.
- [44] K. Ravindranadh, R.V.S.S.N. Ravikumar, M.C. Rao, *J. Non Oxide Glasses*, **2013**, 5, 39-45.
- [45] M.C. Rao, K. Ravindranadh, M.S. Shekhawat, *AIP Conf. Proc.*, **2016**, 1728, 020077 (1-4).
- [46] K. Ravindranadh, R.V.S.S.N. Ravikumar, M.C. Rao, *AIP Conf. Proc.*, **2016**, 1728, 020079 (1-4).
- [47] T. Samuel, K. Sujatha, K. Ramachandra Rao, M.C. Rao, *AIP Conf. Proc.*, **2016**, 1728, 020080 (1-4).
- [48] K. Ravindranadh, D. Sridhar Kumar, K. Durga Venkata Prasad, M.C. Rao, *Int. J. ChemTech Res.*, **2016**, 9(4) 598-603.
- [49] Ch. Srinivasa Rao, M.C. Rao, T. Srikumar, *Int. J. Chem Tech Res.*, **2014**, 6(7), 3935- 3938.
- [50] T. Srikumar, Ch. Srinivasa Rao, M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 6(11), 4697-4701.
- [51] Ch. Srinivasa Rao, T. Srikumar, M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 7(1), 420-425.
- [52] Ch. Srinivasa Rao, M.C. Rao, *Int. J. Chem Tech Res.*, **2015**, 8(2), 524-527.
- [53] M.C. Rao, K. Ravindranadh, *Der. Pharm. Che.*, **2016**, 8(7), 74-79.
- [54] M.C. Rao, *Res. J. Recent Sci.*, **2013**, 2(3), 67-73.
- [55] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Non Oxide Glasses*, **2013**, 5, 39-45.
- [56] K. Parameswara Rao, B. V. Ramesh, Ch. Siva Prasad, M. C. Rao, *Der Pharm. Lett.*, **2016**, 8(9), 341-348.
- [57] Sk. Muntaz Begum, K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *AIP Conf. Proc.*, **2013**, 1536, 27-28.
- [58] P.V. Prasad, K. Ramachandra Rao, M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 7(1), 269-274.
- [59] P.V. Prasad, K. Ramachandra Rao, M.C. Rao, *J. Mol. Struct.*, **2015**, 1085, 115-120.
- [60] S. Rajyalakshmi, B. Brahmaji, K. Samatha, K. Ramachandra Rao, M.C. Rao, *Int. J. ChemTech Res.*, **2016**, 9(1), 7-14.
- [61] M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 6(3), 1904-1906.
- [62] K. Parameswara Rao, B.V. Ramesh, Ch. Siva Prasad, G.V. Ramana, M.C. Rao, *Der Pharm. Lett.*, **2016**, 8(10), 222-228.
- [63] K. Ravindranadh, K. Durga Venkata Prasad and M.C. Rao, *AIMS Mater. Sci.*, **2016**, 3(3) 796-807.
- [64] B. Halliwell, J.M. Gutteridge, *FEBS Lett.*, **1981**, 128(2), 347-352.
- [65] T. Sudha, S. Chidambarampillai, V. R. Mohan, *J. Curr. Chem. Pharm. Sci.*, **2013**, 3(2), 113-122.