

Identification of anti-inflammatory constituents from pet ether extract *Justicia Gendarussa* Burm. f. with GC-MS

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ABSTRACT

Justicia gendarussa Burm.f. is a medicinal plant in Western ghats of Maharashtra in India. The study aims to determine active fractions of *Justicia Gendarussa* for its anti-inflammatory activity and identify their chemical constituents. Physicochemical parameter 5.57 % \pm 0.430 ash value indicate quality and purity of crude drug. It also indicates the presence of inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Sometimes inorganic variables like silica, calcium carbonate, calcium oxalate content of the crude drug can affect total ash value 5.97% \pm 0.212 moisture content WHO guidelines for quality control recommended for less moisture content in crude drug during storage to avoid the bacterial and fungal growth. 14.81 % \pm 0.519 alcohol soluble extractive and 30.91 % \pm 2.220 water soluble extractive. Phytochemical tests showed the presence of alkaloids, steroids and terpenoids, tannins, glycosides, saponins, flavonoids, phenolic compounds and carbohydrates. Elemental analysis by atomic absorption spectroscopy results 61.04 % Fe, 37.14 % Mg, 21.8 % Zn, 10.28% Cu, 2.33 % B in *Justicia gendarussa* Burm.f. Fluorescence analysis gives qualitative assessment some crude drugs with standardization crude drug of *Justicia gendarussa* Burm.f. act differently at different light wavelength. Various constituents of extract showed fluorescence in U.V. range, visible range or in both. The identification of phytoconstituents by GCMS are Caryophyllene, Epiglobulol, 1-Penten-3-one, Hexadecanoic acid, 9, 12, 15-Octadecatrienoic acid, Hexadecanoic acid etc in pet ether extract. As the NIST data Caryophyllene, Hexadecanoic acid and Beta sitosterol showed strong Anti-inflammatory activities.

Keywords : Anti-Inflammatory, Physicochemical Parameter, WHO, GCMS, NIST

I. INTRODUCTION

Herbal medicines have affective treatments on kind of physical and mental health problems, including rheumatism. Contemporary herbal drugs have changed notably from its traditional methods to commercial merchandise being extensively to be had to the general public as over the counter supplements (Zhang Y et.al.). According to reports of worldwide health agency (WHO), Out of overall world's population and about 80 % individual relies on traditional remedy to conform their primary healthcare requirements. From early Nineties, the use

of herbal medicines has steadily extended in Western nations as trading and used extensively by medicine practitioners. Posadzki et al. in 2013 reported that lifetime effect in Western nations were 31% in UK. As per survey of Thompson et al. in 2013 37% in Australia has lifetime effect. Out of 121 medicines come from herbal resources in America, ninety isolated drugs are plant based. Top 25 bestselling drugs in world are originated from the herbal origin. The nonsteroidal anti inflammatory drugs like naproxen and diclofenac, and immunosuppressant like cyclosporine are derived from the herbal resources.

Kali Tilwan belongs to family Acanthaceae with *gendarussa* species. The whole plant has been used in clinical for years as a traditional Indian medicine (TIM). The whole plant is used to treat cold, acute gastric abscess and arthritis by ethanapharmacological practitioner. The previous pharmacological studies demonstrated that the extracts of *Justicia gendarussa* burm f. reported antioxidant and rheumatic properties. Although more than fifty compounds including phenols, triterpenes, long chain hydrocarbons and so on. Only very few chemical components were recognized to be responsible for certain effects. Roots of *Justicia gendarussa* burm f. showed protective effect of *Justicia gendarussa* *Burm.f.* on carrageenan-induced inflammation (Kavitha S K et al). Anti-arthritis potential of the plant *Justicia gendarussa* *Burm f.* on stem (Paval Jaijesh et al.). Anti-inflammatory potential of an ethyl acetate fraction isolated from *Justicia gendarussa* *Burm f.* roots through inhibition of iNOS and COX-2 expression via NF-kB pathway (Kumar et al.).

II. METHODS AND MATERIAL

Collection of plant material carried out from Western Ghats of Sahyadri ranges i.e. Kalsubai and Harishchandragad region. Authentication carried out at BSI, Pune (voucher number: BSI/WRE/Tech/2013/1154). Extraction of dried leaves carried out by Soxhlet hot extraction method followed by preliminary phytochemical screening of crude extracts. The leaves of *Justicia gendarussa* *Burm.f.* were shade dried (3.0 Kg), grinded to powder and then defatting carried out by with pet-ether for 48 h by soxhlet extraction. Pet-ether extract dried under reduced pressure using rotary evaporator (Heidolph Labrota 4000 Efficient, Germany). The colour of extract obtained was dark brown and the percentage yield was 4.50% (w/w). Physicochemical parameter analyzed as like moisture content, water / alcohol soluble extractive values, Total ash value, Acid-

insoluble ash value, Alcohol-soluble extractive value etc. Fluorescence analysis carried out as per the method of Chase and Pratt (1949), the fluorescence analysis was carried out qualitatively. Powdered drug monitored in day light and ultra-violet light (254 nm and 365 nm) with different chemicals. Elemental analysis carried out by AAS. The preliminary phytochemical tests of various extracts of *Justicia gendarussa* *Burm.f.* leaves was carried out. Test for carbohydrates carried out by Molisch test, Fehling's test. Test for glycosides.

Carried out by Keller-Killani test, Borntrager test. Test for proteins carried out by Biuret test, Millon's test. Test for steroids carried out by Salkowski test, Liebermann-Burchard test, Liebermann's test. Test for triterpenes are carried out by Vanillin-sulphuric acid test. Saponins are estimated by foam formation test. Test for alkaloids Dragendorff's test, Mayer's test, Hager's test, Wagner's test. Test for tannins and phenolic compounds are estimated by Ferric chloride test, Dilute nitric acid test etc. The GC-MS analyses were performed by using the TRACE™ 1300 gas chromatography instrument coupled to the mass spectrometer make Thermo Scientific (Model :TSQ 8000). Column (make TG 5MS) 30 m × 0.25 mm dimensions which coated with 0.25 µm film. Carrier gas: Helium gas, with a constant flow rate of 1 ml/min. Hydrogen gas used as fuel. Oven temperature: 60 to 280°C. Thermal Conductivity Detector has temperature: 250°C.

The electron impact mass spectra were measured at acceleration energy of 70 eV. The manual injection of 1.0 µl of the solution of isolated compound was performed in the split mode at a 20:1 split ratio. The interpretation of GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The mass spectrums of unknown compounds were compared with the spectrum of the known components stored in the NIST library and published data. Identification of Phytoconstituent are

carried out by GCMS and compared the data with NIST library. Fingerprinting of phytoconstituents on basis of retention time.

III. RESULTS AND DISCUSSION

A. Physicochemical parameters:

Powdered leaves of *Justicia gendarussa* Burm.f used for physicochemical standardization for estimation of impurities present in crude drug. The different parameters with their results are presented in standardization of the crude drugs the extractive values play a very significant role. With different solvents extraction carried out gives assurance to find different contamination and fatigued constituents e.g. presence of the adulterants water are indicated by alcohol soluble extractive values, substandard processing and poor value of the drug. Ash value gives quality and purity of crude drug. It also indicates the presence of inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Sometimes inorganic variables like silica, calcium carbonate, calcium oxalate content of the crude drug can affects total ash value. WHO guidelines for quality control recommended for less moisture content in crude drug during storage to avoid the bacterial and fungal growth.

Table 1 Physicochemical standardization of the leaves of *Justicia gendarussa* Burm.f.

Sr.No.	Parameter	Values (% w/w)
01	Water soluble extractives	30.91 % ± 2.220
02	Alcohol soluble extractives	14.81 % ± 0.519
03	Total ash value	5.57 % ± 0.430
04	Acid insoluble ash value	2.56 % ± 0.121

05	Water soluble ash value	5.7 % ± 0.231
06	Moisture content (Loss on drying)	5.97 % ± 0.212

B. Fluorescence study

The fluorescence phenomenon shows by plant powder or its extracts. Crude drug may be act differently at different light wavelength. Various constituents of extract showed fluorescence in U.V. range, visible range or in both. Some substances do not show fluorescence phenomenon directly. After decomposition with various reagents to form products showed fluorescence phenomenon.

Table 2. Fluorescence analysis of *Justicia gendarussa* Burm.f. leaf powder.

C. Elemental Detection

Treatment	Visible	UV at 254nm	UV at 365 nm
Powder as such	Dark green	Green	Green
Powder + water	--	Greenish	--
Powder + ethanol	Dark green	Green	Red
Powder + 1N NaOH (aq.)	Yellow	Green	--
Powder + 1N NaOH (alc.)	Light green	Green	--
Powder + 1N HCl	Light green	Green	--
Powder + Conc. HCl	Dark green	Green	--
Powder + 50% HNO ₃	--	Green	--
Powder + Conc. H ₂ SO ₄	Brown	Dark green	Greenish
Sr.No.	Element analyzed	Content in <i>Justicia Gendarussa</i> Burm.f.	

01	Fe	61.04 %
02	Mg	37.14 %
03	Zn	21.8 %
04	Cu	10.28%
05	B	2.33 %

Table 3 Elemental analysis of *Justicia gendarussa* Burm.f.

D. Preliminary phytochemical screening of extracts

The preliminary phytochemical screening of various extracts from the leaves of *Justicia gendarussa* Burm.f. as shown in **Table 4**.

Phytoconstituents	Test	Pet-Ether extract
Alkaloids	Dragendorff's test, Mayer's test, Hager's test, Wagner's test	+
Steroids	Liebermann-Burchard reaction Liebermann reaction Salkowski reaction	+
Triterpenes	Vanillin-Sulphuric acid	-
Tannin and Phenolics	5% FeCl ₃ solution Dilute nitric acid	-
Glycosides	Keller-Killani Borntrager	-
Flavonoids	Shinoda Lead Acetate test NaOH test	-

	Zn/HCl test	
Saponins	Foam test	
Carbohydrates	Molisch's test Fehling's test Benedict's test	-

E. GCMS analysis

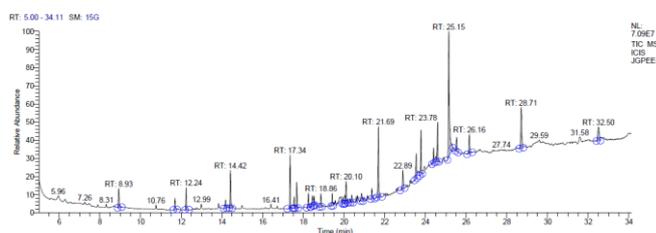


Fig 1. Chromatogram of Pet-ether extract

Table 5. Identified phytoconstituents from extract JGPEE I extract from the leaves of *Justicia gendarussa* Burm.f.

Retention time (Min)	Components	Peak area (%)
8.93	3-Dodecene-E	2.51
12.24	Caryophyllene	2.13
14.42	Epiglobulol	4.41
17.35	1-Penten-3-one	5.18
18.45	Hexadecanoic acid	2.08
20.10	9,12,15-Octadecatrienoic acid	3.28
21.69	2- Heptanone	8.79
23.54	Spiro [4,5] decan-7-one	3.31
23.78	2-Pentanoic acid	5.12
24.59	2-Pentanoic acid	4.60
25.15	Hexadecanoic acid	21.86
26.16	Tetracontane	2.80
32.50	Beta sitosterol	3.76

IV. CONCLUSION

Physicochemical parameter $5.57 \% \pm 0.430$ ash value indicate quality and purity of crude drug. It also indicates the presence of inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Sometimes inorganic variables like silica, calcium carbonate, calcium oxalate content of the crude drug can affects total ash value $5.97\% \pm 0.212$ moisture content WHO guidelines for quality control recommended for less moisture content in crude drug during storage to avoid the bacterial and fungal growth. $14.81 \% \pm 0.519$ alcohol soluble extractive and $30.91 \% \pm 2.220$ water soluble extractive.

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V. Acknowledgement

The authors are always remaining acknowledged with Dr. Sanjay Malpani (Chairman, SP Sanstha's) Dr. Anil Rathi (Secretary, SP Sanstha's) for availability of laboratory and facilities at Sangamner College.

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Cite this article as :

Keshav K. Deshmukh, Narendra D. Phatangare, "Identification of anti-inflammatory constituents from pet ether extract *Justicia Gendarussa* Burm. f. with GC-MS", *International Journal of Scientific Research in Science and Technology (IJSRST)*, Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 5 Issue 4, pp. 52-57, ETCES-2019, January 2019.
Journal URL : <http://ijsrst.com/IJSRST195412>