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Phytochemical Screening and FTIR analysis of Indian *Aegle marmelos L.* (Bael) Extracts.

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Abstract

The present study was made to investigate the phytochemical screening on leaf extract of Indian *Aegle marmelos L.* (Bael). The physical parameters determined were pH, total ash, acid soluble ash, acid insoluble ash value, water extract, methanol extract, chloroform extract , petroleum ether extract & UV fluorescence Test. The phytochemical analysis were also performed & the results of phytochemical screening of the aqueous leaves extract revealed the presence of alkaloids, flavonoids, proteins, tannin & Phenolic compound. The different parts of Bael are used for various therapeutic purposes, such as for treatment of asthma, anaemia, fractures, healing of wounds, swollen joints, high blood pressure, jaundice, diarrhoea, controlling diabetes, decoction is given to patient suffering from fever and cold. A dried powdered leaves sample were dissolved in different reagents & observed under UV light and fluorescence were recorded. The FTIR Spectra of water Extract, Methanol Extract & ether extract were also performed. The present study clearly indicates that the compounds like alkaloids, flavones, terpenoids and saponins are the active principles present in the leaves of Beal. The FTIR spectra analysis gives an idea about the different functional groups were present in this sample and it can be isolate and can be use the active components of this natural plant for further drug preparation.

Key words: water extracts, methanol extract, UV fluorescence, alkaloids, flavonoids, Proteins, tannin

Introduction

Biological active compounds from natural sources have always been of great interest to scientist working on infectious diseases[1].The medicinal value of plants lies in some chemical substances that produce a definite physiological action on human body. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious disease. The most important of these are flavonoids, tannins and Phenolic compound. It is a well known fact that plant produces various chemicals for self defence. [2]

Indian *Aegle marmelos L.* (Bael Aegle marmelos (Bael) is a holy tree of India, from the Rutaceae family and **Kingdom** Plantae, It is commonly known as the bael fruit, Indian bael, holy fruit, golden yellow apple tree in English, as Bilva, Sriphal, or Shivadruma (the tree of Shiva) in Sanskrit, and Bael,baelputri, bela,in Hindi[3]

Fig No 1 & 2 . Indian *Aegle marmelos L.* Bael Leaves

Geographical distribution: It is an indigenous tree found in Iran, India, Myanmar, Pakistan, Bangladesh and most of Southeast Asian countries.[4] Bael tree is native to India and is found growing wild in Sub-Himalayan region & found in other states in India like Himachal Pradesh, Andhra Pradesh, Bihar, Jammu and Kashmir, Kerala, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Uttar Pradesh, Tamil Nadu and West Bengal.8It is also found in Bangladesh, Pakistan, Sri Lanka, Thailand ,Egypt & Malaysia [5] is a medium sized tree having profuse dimorphic branches, alternate, trifoliate and deep green leaves, membranous leaflets, large sweet scented, greenish white flowers, large and globosely fruits[6]

Medicinal Properties: Different medicinal system like Siddha, Unani and Ayurvedic systems provide information about the potential effects of bael [7].This is an important medicinal plant having traditional and folk medicines and ethno medicinal applications. For diarrhoea and dysentery treatment, bael fruit is having traditional application. This plant leaves are the causes of abortion or infertility in women.[8]Bael can be considered as an important medicine in Ayurveda for treating chronic diarrhea, dysentery, brain tonic, etc. A good combination of five parts of bael such as root, bark, leaf, flower and fruit can be highly considered as an effective agent for the treatment of certain mental disorders. Fruit powder of bael produces anti-cancer activity.[9]The leaves are supposed to reduce bowel complaints, bleeding piles, diarrhea, and dysentery.[7]Its leaf extract is used to cure ophthalmic, ulcer and intestinal worms by twice daily intake.[10]Treatment of eye diseases requires poultice that are obtained from bael leaf.[11] Leaf juice is having a number of medicinal importance especially for controlling diabetes. Its root decoction is given to patient suffering from fever and cold The different parts of Bael are used for various therapeutic purposes, such as for treatment of asthma, anaemia, fractures, healing of wounds, swollen joints, high blood pressure, jaundice, diarrhoea healthy mind and brain typhoid troubles during pregnancy[12] The aqueous extracts of the stem and root bark are used to treat malaria, fever, jaundice and skin diseases such as ulcers[13] Essential oil isolated from the leaf has antifungal activity [14] Various phytochemical and biological evaluations have been reported as anti-diabetic[15]antioxidant,antithyroid[16] Antidiabetic property, antidiarrhoeal activity, antiulcer activity of seeds, antifungal activity of leaves and antitumor and antimutagenic activity of this plant are clinically evaluated[17]Different organic extracts of the leaves of A. marmelos have been reported to possess alkaloids,

cardiac glycosides, terpenoids, saponins, tannins, flavonoids and steroids [18, 19]. Aegle marmelos fruit pulp reported for the availability of steroids, terpenoids, flavonoids, Phenolic compounds, lignin, fat and oil, proteins, carbohydrates, alkaloids, cardiac glycosides and flavonoids [20].

Materials and Methods:

Sampling and Collection of leaves: The sample of Beal leaves was collected from the Himayat Bagh of Aurangabad M.S India . It was washed 2-3 times with d/w and air dried in open air. The dried leaves were powered in mixer grinder and was kept in plastic bag untill it was analysed. All the chemicals used in analysis was AR grade . All the Apparatus used was first washed with d/w. The physical parameters includes pH, total ash ,acid soluble ash ,acid insoluble ash, water extraction, methanol extraction, chloroform extract ,petroleum ether extract, Fluorescent test was done with the crude leaf powder by the procedures given in the Ayurvedic pharmacopoeia of India . FTIR screening was carried out by using aqueous extract, chloroform & methanol extract. The preliminary phytochemical analyses was done by using water extraction , methanol extract,Chloroform extract and petroleum ether extract followed by the standard procedures [20,21].

Determination of pH range : 5gm of the sample weighted and immersed in 100ml of water in a beaker and the pH of the formulation was determined using a calibrated pH meter. [18] as shown in table no 1

Determination of Total ash: Accurately 5 gm of sample was taken in finely clean & previously weighed silica crucible and ignited for 3-4 hrs with gradually increasing in temperature up to 500^{o}C. After ignition of leaves crucible was cooled in a desiccator and weighed as total ash. The ash was used to determine the acid soluble ash and acid insoluble ash.

Determination of Acid insoluble ash: The ash was dissolved in 2N hydrochloric acid in the beaker. Stirred well for the digestion of ash and filtered through whatmann filter paper number 41. The residue remains after filtration is ignited in clean silica crucible by gradually increasing temperature up to 500^{o}C. The crucible with the residue was cooled in desiccators and weighed. The residue remains after ignition was calculated as acid insoluble ash in percentage. From this calculation the acid insoluble ash was calculated by taking the difference between the Total ash & acid insoluble ash in percentage as shown in table no 2

Fluorescence Analysis: The Fluorescence property of the sample was observed both in visible and ultra-violet light for their fluorescence characters (short wave length 254nm and long wave length 365nm) powered leaf sample was taken with various solvents and florescence property was noted . [19]as shown in table no 3

Water extraction: Accurately weight 10gm of sample was introduced into the 500ml round bottom flask with 100ml of double distilled water. The sample was refluxed on flame for six hrs. The sample was cooled and filtered by the suction pump. The excessive water was evaporated for the preservation of the sample and it was kept at 40C for 12 hrs. The percentage of the extracts was calculated as shown in table no 4

Microwave Extraction (Methanol):10 gm of the sample was kept in the clean round bottom flask. Approximately 100ml methanol was used as a solvent for extraction0. The sample was refluxed by microwave radiations using microwave oven for 20-30 min. at 50% power, 420 watt and 150^{o}C. The sample was cooled

and filtered by the suction pump. The excessive solvent was evaporated & it was kept for 12hrs.The extract was weighed and calculated in percentage as shown in table no 4

Chloroform and petroleum ether extracts: 10 gm of the sample was kept in the two clean beaker 100ml each solvent was added in each beaker respectively. The sample was soaked in solvent for 12hr at room temperature. It is than filtered .The excessive solvent was evaporated. The extract was weighed and calculated in percentage as shown in table no 4

Preliminary phytochemical screening: The aqueous, Chloroform, methanol and petroleum ether extracts were used for preliminary phytochemical analyses using standard procedures [20,21].

a)Test for alkaloids:

Wagner's test: About 10 mg of extract was taken and few drops of Wagner's reagent were added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

b)Test for Flavonoids: Shinoda Test:10mg of extract was added to pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid was added. Formation of pink colour indicates the presence of Flavonoids.

c) Test for Phenols & Tannins:

Lead acetate test: 10mg of extract was taken and 0.5 ml of 1%leadacetate solution was added & the formation of precipitate indicates the presence of tannin and Phenolic compounds.

Ferric chloride test: 5mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black colour indicates the presence of tannins.

Sodium hydroxide test: 5mg of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue which indicates the presence of phenols.

d) Test for steroids and sterols:

Salkowski's test: 5mg of extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound in the extract.

e) Test for carbohydrate: Fehling's test: 5ml of Fehling's solution is a water bath. The formation of yellow or rest for precipitate indicates the presence of reducing power.

f) Test for Saponins: **Foam test:** 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for15 min. The formation of foam to a length of 1cm indicated the presence of saponins

g) Test for Glycosides: Glycoside test: 0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow colour indicates the presence of glycosides.

h)Test for Protein & amino acids: Biuret test: To 0.5 mg of extract equal volume of 40% NaOH solution and two drops of 1% copper sulphate solution was added. The appearance of violet colour indicates the presence of protein.

Ninhydrin test: About 0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

i) Test for Anthraquinone: Borntragers test :About 0.5 gm of the extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate was shaken with equal volume of 10%ammonia solution. A pink violet or red colour in the lower layer indicates the presence of anthraquinone.

Mayer's Test: 5ml of stock solution and add few drops of Mayer's reagent then formed white and creamy precipitate in the tube.

Result & Discussion: Table no 1: Determination of pH of Indian Bael Leaves

Sr. No.	Parameter	Result
1	pH meter value	6.14

Table no 2: Determination of different ash values of Indian Bael Leaves

Sr. No.	Parameter	Result
1	Total Ash value	4.7%
2	Acid soluble Ash value	1.6%
3	Acid insoluble Ash value	2.7%

Fig no 3 Acid Insoluble ash and Total ash

Fig no:4 Graph of ash value

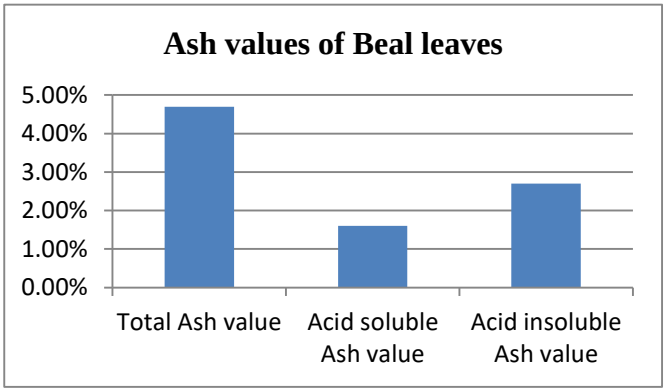

Table no 3 Qualitative determination of fluorescent test of Indian Bael Leaves

Sr. No.	Reagent	Visible Light	Long Wave length	Short Wave Length
	Dry powder	Green	Greenish brown	White
1.	Powder sample + Distilled water	Light Green	Yellowish green	Brownish
2.	Powder sample +chloroform	Green	Dark green	Yellow green
3	Powder sample + Ammonia	Dark green	Dark green	Black
4	Powder sample + Glacial acetic acid	Yellowish green	Dark green	Black
5	Powder sample + Ethyl alcohol	Light green	Green	White layer on the surface
6	Powder sample + Acetone	Yellowish green	Dark green	Upper layer is white & lower layer is black
7	Powder sample + dil H_2SO_4	Green	Greenish Black	Black
8	Powder sample + dil HCL	Dark brown	Dark brown	Black
9	Aqueous NaOH solution	Yellow green	Dark green	Yellowish

Table no:4 Quantitative determination of Indian Bael leaves extract in Various solvents

Sr. No.	Parameter	Result
1.	**Water extract**	**28.5%**
2.	**Microwave extract of methanol**	**19.1%**
3.	**Chloroform extract**	**11.3%**
4.	**Petroleum ether extract**	**6.8%**

Fig no: 5 Graph of Various extracts of beal leaves

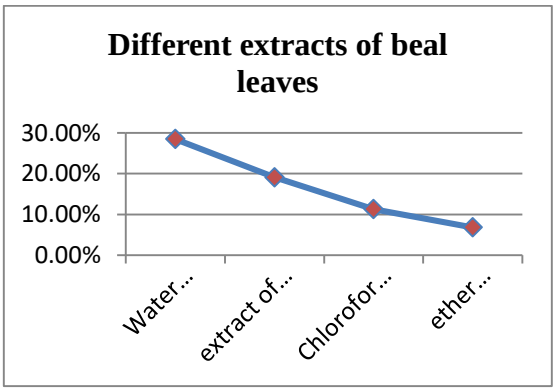

Table no 5. Preliminary phytochemical screening of Indian Bael Leaves

Plant constituent	Extracts				
	Chloroform Extract	Methanol Extract	Aqueous Extract	Petroleum Ether Extract	Name of Test
i) Alkaloids	+	+	+	+	Wagner's test
ii) Flavonoids	+	+	+	+	I) Lead acetate test
	+	+	+	-	II) Shinoda test
iii) Test of Phenols & Tannins	+	+	+	-	I) Lead acetate test
	+	+	+	+	II) Ferric chloride test
	+	-	-	-	III)Sodium hydroxide test
iv) Steroids & Sterols	-	-	-	-	Salkowski's test
v) Carbohydrates	+	+	+	+	I) Fehling's test
	+	+	-	-	II) Benedict's test
vi) Saponins Test	+	+	+	+	I) Honey comb test
	+	-	+	+	II) Foam test
vii) Glycosides	-	-	-	-	Glycosides test
viii) Proteins & amino acid	+	-	-	-	I) Biuret test
	+	+	+	-	II) Ninhydrin test
ix) Anthraquinone Test	+	+	+	-	Borntrager's test
x) Phlobatannins	-	-	-	-	--
xi) Mayer's Test	-	-	-	-	--

Positive : +, Negative: -

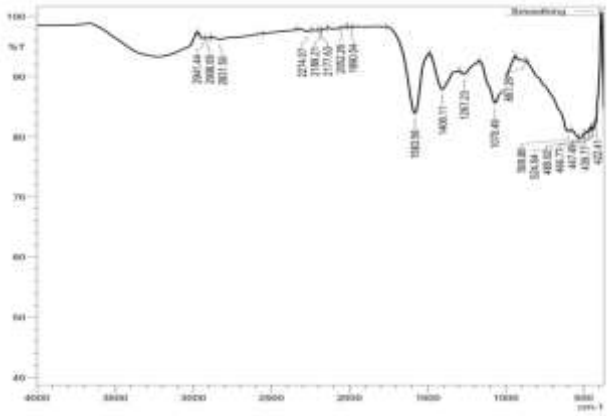

Fig no 3. FTIR spectra of water extract

Tab le no. 6 FTIR spectra Analysis of water extract of Beal leaves

Sr no.	Frequency cm^{-1}	Bond	Functional group
1.	2941.44	C-H stretch, medium	Alkane
2.	2908.65	C-H stretch, medium	Alkane
3.	2831.50	C-H stretch, medium	Alkane
4.	2274.07	C≡C stretch ,medium	Alkynes
5.	2052.26	C≡C stretch ,medium	Alkynes
6.	1583.56	C=C stretch	Cyclic Alkenes
7.	1406.11	C-H stretch, Bending	Methyl group
8.	1070.49	C-O stretch, medium	Alcohol
9.	599.86	C-Br stretching	Halogen compound
10.	524.64	C-Br stretching	Halogen compound
11.	422.41	C-I stretching	Halogen compound

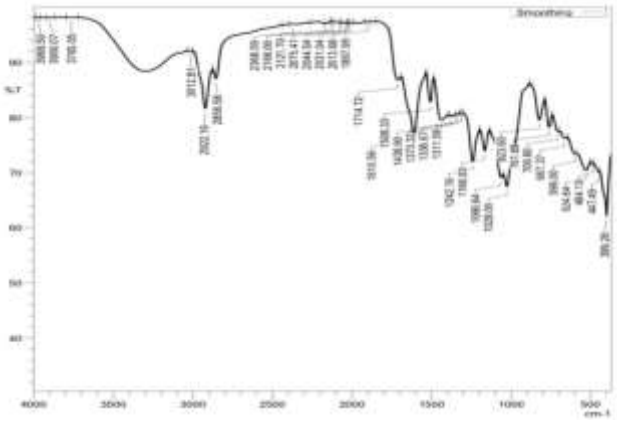

Fig no 4. FTIR spectra of Methanol extract.

Table no. 7 FTIR spectra Analysis of Methanol extract of Beal leaves

Sr no.	Frequency cm^{-1}	Bond	Functional group
1.	2922.16	C-H stretch, medium	Alkane
2.	2856.58	C-H stretch, medium	Alkane
3.	1714.72	C=O stretch, medium	Carbonyl group
4.	1610.56	C=C stretch ,medium	Aromatic ring
5.	1508.33	C=C stretch ,medium	Cyclic Alkenes
6.	1311.59	C=C stretch	Cyclic Alkenes
7.	1243.16	C-O stretch, Bending	Alcohol
8.	1066.64	C-O stretch, medium	Alcohol
9.	761.88	C-Br stretching	Halogen compound
10.	524.64	C-Br stretching	Halogen compound
11.	447.49	C-I stretching	Halogen compound

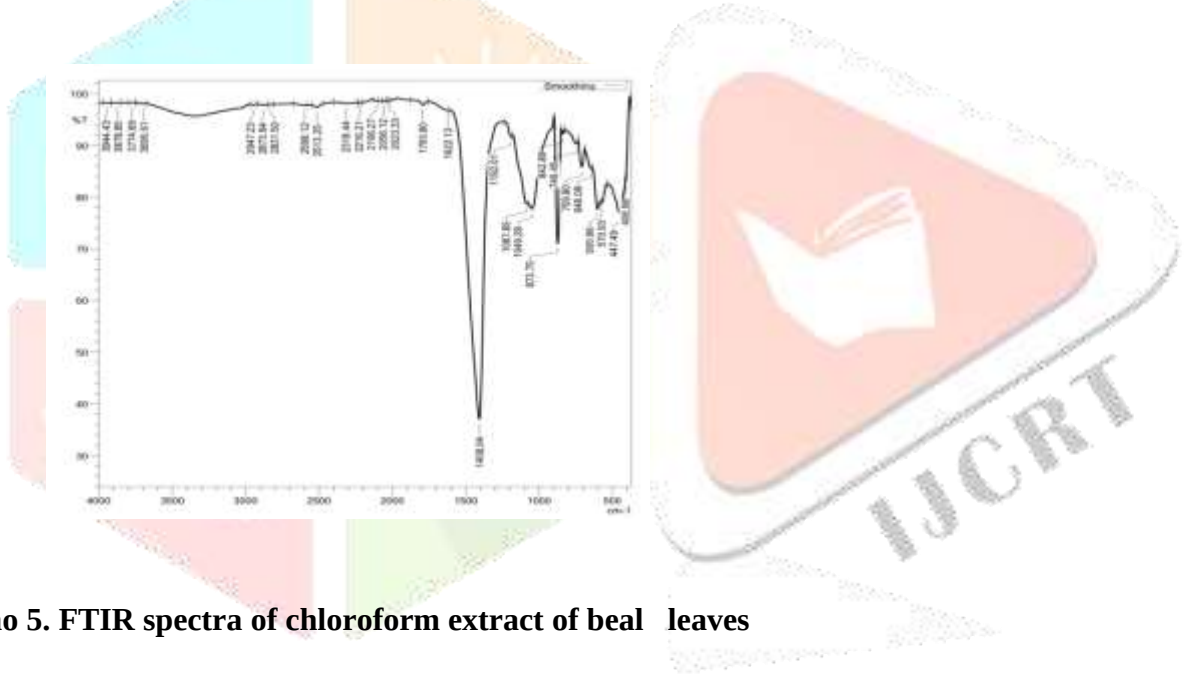

Fig no 5. FTIR spectra of chloroform extract of beal leaves

Table e no. 8. FTIR spectra Analysis of chloroform extract of Beal leaves

Sr no.	Frequency cm^{-1}	Bond	Functional group
1.	3695.61	O-H Stretch	Carboxylic acid
2.	2947.23	C-H stretch, medium	Alkane
3.	2856.58	C-H stretch, medium	Alkane
4.	1793.80	C=O stretch, medium	Carbonyl group
5.	1622.13	C≡C stretch ,medium	Alkynes
6.	1408.04	C=C stretch ,strong	Cyclic Alkenes
7.	1192.01	C=C stretch	Cyclic Alkenes
8.	1049.28	C-O stretch, Bending	Alcohol
9.	873.75	C-Cl stretch	Halogen compound

10.	**709.80**	**C-Br stretch, medium**	**Halogen compound**
11.	**599.86**	**C-Br stretching**	**Halogen compound**
12.	**447.49**	**C-I stretching**	**Halogen compound**
13.	**406.98**	**C-I stretching**	**Halogen compound**

Physical analysis: The Beal leaves sample was tested for various physical properties such as (pH ,total ash, acid soluble ash, Acid insoluble ash, water extract, methanol extract, chloroform extract, petroleum ether extract & florescence test) were determined as shown in Table no 1,2,3 & 4

The pH was observed were 6.14

The total ash content in sample indicates amount of inorganic oxides present in it. The total ash was found in Beal leaves were 4.7% , this may be due to the fact that we have taken its leaves for estimation. The acid soluble ash was found were 1.6% & acid insoluble ash value were 2.7%. (Table no.2)

Fluorescent Analysis: In a sample when distilled water was added powdered sample of beal it was observed in ultraviolet light it showed light green colour in visible light and dark green colours was observed in long wavelength and white colour was observed in short wavelength as summarized in following table shown in table no 3 different solvent were used to determine the fluorescent analysis and it showed different colours in different in long wavelength and short wavelength

The water extract of leaves of these samples (Table 4) shows that, maximum water extract is obtained 28.5% and minimum in petroleum ether 6.8%

Methanol Extraction: The methanol was found 19.1%

Chloroform extract was found 11.3% & **petroleum ether** extract was found 6.8% shown in table no 4 **water extract<methanol extract< chloroform extract< petroleum extract.**

Phytochemical Test: Phytochemical are the plant nutrient which act as secondary metabolized.

Alkaloids were present in chloroform extracts, aqueous extracts and methanol extract & petroleum ether extracts when performed by Wagner's test.

Flavonoids were only present in methanol extract, aqueous extract when tested by Lead acetate test.

Phenolic and Tannins was present in all the extracts by using ferric chloride test.

Steroids and Sterols was found absent in all the extracts

Carbohydrates was absent in the petroleum ether extract and found present in all other extract when tested by Fehling's method and Benedict's method.

Saponins test was found present in chloroform extracts, aqueous extracts and methanol extract & petroleum ether extracts when tested by foam test.

Glycosides were found absent in all the extracts when tested by glycosides test.

Portions and Amino acid were present in chloroform extract,
Methanol extract, aqueous extract when tested by Ninhydrin Method and but found absent in petroleum ether extract.

Anthraquinone test were present in chloroform extract, Methanol extract, aqueous extract and found absent in ether extract when tested by Borntragers test.

Phlobatannins and Mayer's test were absent in the all extract.

FTIR spectra Analysis shown different peaks of chemical constituents present in it as shown in table no6, 7 & 8.This spectra analysis gives an idea about the different functional groups were present in this sample and it can be isolate the active components of this natural plant for further drug preparation

Conclusion:. The present study clearly indicates that the compounds like alkaloids, flavones, terpenoids and saponins are the active principles present in the leaves of Beal.. This spectra analysis gives an idea about the different functional groups were present in this sample and it can be isolate and can be use the active components of this natural plant for further drug preparation. Hence more research can be done to investigate the unexplored potential of this Indian medicinal plant.

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