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Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Phytochemical analysis of Indian folklore medicinal plants*Cassia fistula* and *Luffaacutangula*

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Abstract : Inflammation is a body defence reaction to prevent the spread of injurious agent and to remove the necrosis cells and tissues. Inflammatory abnormalities are a large group of disorders which underlie a vast variety of human diseases. During treatment of inflammatory diseases, many conventional therapies (non-steroidal anti-inflammatory drugs) used to relief pain and inflammation. Continuous use of the intended drugs is frequently associated with serious side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents with a better safety profile. *Cassia fistula* Linn.(Leguminosae) and *Luffaacutangula* Linn. (Cucurbitaceae) has many therapeutic uses mentioned in Ayurveda. This work considers about the phytochemical analysis of the above plants to study its anti-inflammatoryactivity.
Keywords: Phytochemistry, flavonoids, terpenoids, tannin, steroids, C*assia fistula, Luffaacutangula*,

1. Introduction

Since thousands of years back, plants are used as a major source for medicine as they found to possess a reservoir of bioactive compound[1]. Plants have been a common source of medicinal property, either in the form of pure active compound or as traditional preparations and it are reasonable to use local plants[2]. Identifying on the plant phytochemistry provides a fundamental use of plants as storage of chemical agents in the field of medicine[3].

The phytochemical analyses of plants from *Cassia fistula* and *Luffa acutangula* were studied extensively. Identifying the importance of secondary metabolites in the field of medicine, the presence of tannins, phlobatanins, saponins, flavonoids, terpenoids, glycosides and steroids was detected. Folklore medicine are widely used in our ancient period[30].

Cassiafistula Linn. (Leguminosae) isanornamental plant widelycultivatedthroughoutIndia and commonlycalled as Sarakondraiin Tamil.*Cassia fistula* plant have naturally occurring bioactive compounds and are mostly secondary metabolites which are now a days being used as medicines, dietary supplements and other

useful commercial products[25]. Also, has been reported to contain anthraquinone the principal laxative constituent of many plants used as purgative[26].

*Luffaacutangula*is a species of *Luffa*. It is commercially known for its unripe fruits as a vegetable. *Luffaacutangula* (ridge gourd) belongs to the family cucurbitaceae is used as fruits and vegetables, and have considerable economic value[27]. The fruits of cucurbits are very useful in terms of human health, i.e. purification of blood, remove of constipation, good for digestion, and give energy [28].The juice of the fresh leaves is dropped in to the eyes of children in granular conjunctivitis, also to prevent the lids adhering at night from excessive meibomian secretion[29].

2. Materials and Methods

The present study was carried out the phytochemistry of *Cassia fistula* and *Luffaacutangula*. Qualitative and Quantitative analysis were done and the ethanolic extracts of the two plant species was used for GC-MS studies. The details of the material used and methods followed are described below.

2.1 Collection of Plant Materials

Fruit pulp of *Cassiafistula* and *Luffaacutangula* were collected in the month of Jan-Feb, 2013 from local areas of Thiruvannamalai (Ramanaashramam). Fruit pulp of *Cassia fistula* were dried and finely powered. Fresh fruits of *Luffaacutangula* were crushed and used for the study.

2.2 Chemicals and Reagents

Ethanol, Fehling's reagent, Hydrochloric acid, sulphuric acid, Ferric chloride, acetic anhydride, chloroform, glacial acetic acid, ammonia, magnesium, Anthrone reagent, Bradford reagent, Mayer's reagent.

2.3 Extraction

The plant materials were powdered and 30gm of powder sample was extracted with 150ml of ethanol (1:5) by using soxhlet apparatus [Fig. 1]. The whole apparatus was kept over a heating mantle and was heated continuously for 24 hours at boiling point of solvent. The extract of *Cassia fistula*[Fig. 2] and *Luffa acutangula*[Fig. 3] was concentrated to dryness and the residues were transferred to a pre weighed sample bottle and were stored in a desiccator for further studies.

Fig 1: Soxhlet apparatus

Fig 2: *Cassia fistula* Extract

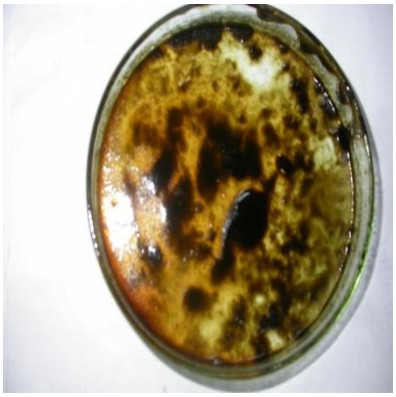

Fig 3*: Luffa acutangula* Extract

2.4 Qualitative Analysis

Different biochemical parameters like reducing sugar, Flavonoid, Terpenoid, Tannin, Saponin, Anthraquinone, glycosides, alkaloids etc. were tested.

2.4.1. Test for Reducing Sugar

The aqueous extract was added to boiling Fehling solution in a test tube, a brick red colour indicates the presence of reducing sugars.

2.4.2. Test for Flavonoids

The extract and add a few magnesium turnings, followed by the addition of conc. HCl drop by drop. Pink colour indicates the presence of flavonoids.

2.4.3. Test for Steroids Ad Terpenoids

Extract, dry and dissolve in chloroform. Add a few drops of acetic anhydride and conc.H_2SO_4 and keep undisturbed for few minutes. Formation of green colourindicates the presence of steroids, while pink colour indicates the presence of terpenoids.

2.4.4. Test for Tannins

To extract, add 2 drops of 5% $FeCl_3$. Presence of dirty green precipitate indicates the presence of tannin.

2.4.5. TEST FOR SAPONIN

To extract was shaken with 5ml of distilled water and was heated to the boiling point. Frothing indicates the presence of saponin.

2.4.6. Test for Anthraquinones

To powdered material add 10 ml of 1% HCl and boiled for 5 minutes. Filter the sample and allowed to cool. Partition the cool filtrate against equal volume of chloroform. Carefully transfer the chloroform layer into clean test tubes. Shake with equal volume of 10% ammonia solution and allow the layer to separate. Presence of delicate rose pink colour indicates the presence of combined anthraquinones.

2.4.7. Test for Glycosides

To 0.5 gm of extract diluted to 5ml with distilled water and add 2ml of glacial acetic acid and containing one drop of ferric chloride solution .This was underplayed with 1ml of conc. H_2SO_4. Brown ring at the interface the presence of glycosides.

2.4.8. Test for Alkalods

5 ml of extract evaporated to dryness. Residue heated on a boiling water bath with 2% HCl. Then filtered, treated Mayer's reagent. Yellow precipitate indicates the presence of alkaloid.

2.5 Quantitative Test for Cassia Fistula

2.5.1 Determination of Moisture

5 gm of material was taken in a pre-weighed petridish. The petridish was placed without lid into an oven at 110^0C for three hours. The petridish was taken out and closed immediately with a lid. The dish was cooled in a desiccator and weighed. The amount of moisture of the material was calculated from the difference in weight.

2.5.2 Estimation of Carbohydate

Weighed amount of fresh tissue was homogenized with distilled water. The homogenate was filtered using a two layered cheese cloth. The filtrate was then centrifuged at 10,000rpm for 15min. The supernatant was collected and the volume was made up to 25ml using distilled water. An aliquot of sample was pipetted out and 4ml Anthrone reagent added. It was then kept in a boiling water bath for 10 min. The tubes were cooled and the absorbance was measured at 530nm. The amount of total carbohydrate present was determined using the standard graph of glucose.

2.5.3 Estimation of Protein

Total protein present in the plant was estimated by Lowrey's method. 1gm powdered plant material was homogenized in 5ml of 0.1 M PO_4 buffer. The homogenate was filtered through double layered cheese cloth and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and the volume was made up to 1.5ml by PO_4 buffer. After that 1.5ml of Bradford reagent was added and kept it for 5 minutes. The absorbance was recorded spectrophotometrically by using appropriate blank at 595nm.The protein content was calculated from the standard graph of BSA or Bovine Serum Albumin.

2.6 GC-MS Analysis

2.6.1 GC-MS

Gas chromatography–mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different Substances within a test sample.

The primary goal of instrument analysis is to quantify an amount of substance. This is done by comparing the relative concentrations among the atomic masses in the generated spectrum. Two kinds of analysis are possible, comparative and original. Comparative analysis essentially compares the given spectrum to a spectrum library to see if its characteristics are present for some sample in the library. This is best performed by a computer because there are a myriad of visual distortions that can take place due to variations in scale. Computers can also simultaneously correlate more data (such as the retention times identified by GC), to more accurately relate certain data.

Another method of analysis measures the peaks in relation to one another. In this method, the tallest peak is assigned 100% of the value, and the other peaks being assigned proportionate values. All values above 3% are assigned. The total mass of the unknown compound is normally indicated by the parent peak. The value of this parent peak can be used to fit with a chemical formula containing the various elements which are believed to be in the compound. The isotope pattern in the spectrum, which is unique for elements that have many isotopes, can also be used to identify the various elements present. Once a chemical formula has been matched to the spectrum, the molecular structure and bonding can be identified, and must be consistent with the characteristics recorded by GCMS.

Typically, this identification done automatically by programs which come with the instrument, given a list of the elements which could be present in the sample

2.6.2 Preparation of Extract

2 µl of the ethanolic extract of *Cassia fistula and Luffaacutangula*was employed for GC/MS analysis.

2.6.3 Instruments and Chromatographic Conditions

GC-MS analysis was carried out on a GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising an AOC-20i auto sampler and

Gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions:

2.6.3.1 GC Programme

- Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 x 0.25mm x 0.25µm df
- Equipment: GC Clarus 500 Perkin Elmer
- Carrier gas: 1ml per min, Split: 10:1
- Detector: Mass detector Turbo mass gold-Perkin Elmer
- Software: Turbomass 5.2
- Sample injected: 2µl

2.6.3.2 Oven Temperature Programme

- 110° C -2 min hold
- Up to 200° C at the rate of 10 ° C/min-No hold
- Up to 280 ° C at the rate of 5° C / min-9 min hold
- Injector temperature 250° C
- Total GC running time 36 min

2.6.3.3. MS Programme

- Library used NIST Version-Year 2005

- Inlet line temperature 200° C
- Source temperature 200 ° C
- Electron energy: 70 eV
- Mass scan (m/z): 45-450
- Solvent Delay: 0-2 min
- Total MS running time: 36 min

3. Results

3.1 Extraction

The phytochemicals present in the plant material was extracted by the distillation method using soxhlet apparatus. The solvent, ethanol was used for the separation of chemical component.

3.2 Phytochemical Analysis

Standard phytochemical screening for flavonoid (Ferric chloride test), glycosides (Fehling's test), alkaloids (Mayer's test), tannin (Ferric chloride test), saponins (foam test) were done. (Harborne, 1973).

Table 1: Phytochemicals of *Cassia fistula*

Test	Test method	Test Result
Flavanoid	Shinoda test	+
Glycoside	Killer-Killiani Test	+
Alkaloid	Mayer's test	+
Tannin	Ferric chloride	+
Saponin	Foam test	+
Anthroquinone		+
Steroids	Libermann-Burchard Test	+
Terpenoids	Libermann-Burchard Test	+
Reducing sugar	Fehling's test	+

Table 2: Phytochemicals of *Luffaacutangula*

Test	Test method	Test Result
Steroid	Libermann-Burchard Test	+
Tannin	Ferric chloride test	+
Flavanoid	Shinoda test	+
Alkaloid	Mayer's test	-
Glycoside	Killer-Killiani Test	-
Anthroquinone		+

The qualitative phytochemical investigations of *Cassia fistula* [Table 1]and *Luffaacutangula* [Table 2] extract showed the presence of steroids, flavonoids, saponins, alkaloids and tannin in the ethanol extracts, saponin is not present in *Luffaacutangula*extract.Result shows that in *Cassia fistula* the moisture content of plant was found to be 90%, while the least content was found to be phenol which was only about 0.002mg/g fresh tissue.

The carbohydrate content is 8.35mg/g and phenol, protein, content is 0.0024, 1.94, mg/g respectively. Biochemical parameters such as protein, carbohydrate, phenol were analyzed and the results were given [Table 3].

Table 3: Quantitative analysis of cassia fistula

Phytochemical compounds	Amount present
Carbohydrate	83.5mg/gm L
Protein	1.94mg/gm L
Moisture content	90%

3.2.1 Comparison of Phytochemical Constituents of *Cassia Fistula* and *Luffaacutangula*

Based on the test results *Cassia fistula* contains more phytochemical constituents when compared to *Luffaacutangula*. The results are shown below [Table 4].

Table 4: Phytochemical constituents of *Cassia fistula* and *Luffaacutangula*

Test	Test Method	*Cassia fistula*	*Luffaacutangula*
Flavanoid	Shinoda test	+	+
Glycoside	Killer-Killiani Test	+	-
Alkaloid	Mayer's test	+	-
Tannin	Ferric chloride	+	+
Saponin	Foam test	+	-
Anthroquinone		+	+
Steroids	Libermann-Burchard Test	+	+
Terpenoids	Libermann-Burchard Test	+	Nil
Reducing sugar	Fehling's test	+	Nil

3.3 GC-MS Results

3.3.1compounds Identified: *Luffa Acutangula*

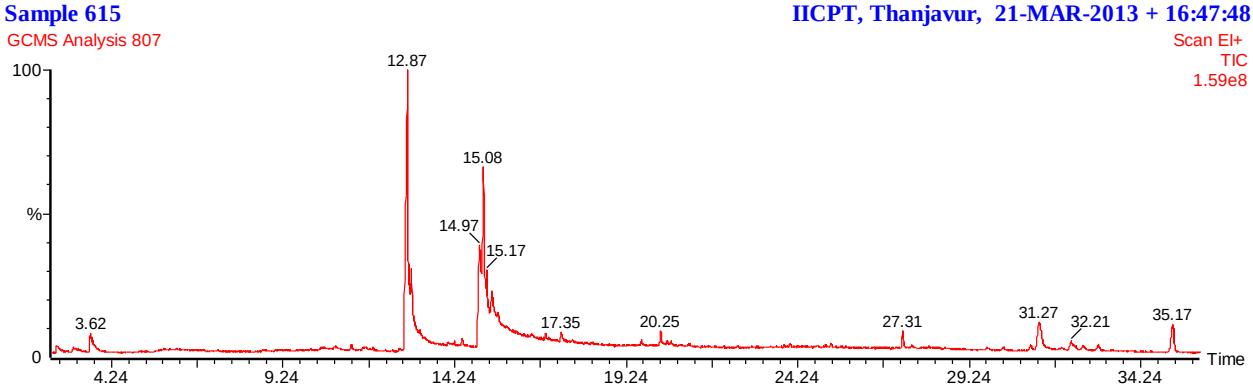

Fig 4: GC-MS chromatogram –*Luffaacutangula*

GC-MS chromatogram analysis of the ethanolic extract of *Luffaacutangula* showed[Fig 4] ten peaks which indicating the presence of ten phytochemical constituents. On comparison of the mass spectra of the constituents with the help ofDr Duke's Phytochemical and Ethanobotanical databases[22], the ten phytocompounds were characterized and identified. The various phytochemicals which contribute to the medicinal activities of the plant were shown. The mass spectra of all the phytochemicals identified in the whole plant ethanolic extract of *Luffaacutangula*were shown. Of the ten compounds identified, the most prevailing compounds were 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 9,12,15-Octadecatrienoic acid, (Z,Z,Z), 9,12,15-Octadecatrienoic acid, (Z,Z,Z), 1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E). Among the compounds, three compounds were reported to have 5 Alpha reductase inhibitoractivity and other three compounds were reported to have anti-microbial activity in general in general, and no activity was reported in Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl), Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á) of the sample showed in [Table 5].

Table 5: Components identified in the sample –*Luffaacutangula* [GC- MS study]

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	3.62	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	3.10
2	12.87	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	36.91
3	14.97	9-Octadecynoic acid	$C_{18}H_{32}O_2$	280	14.49
4	15.08	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	19.85
5	15.17	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	9.55
6	20.25	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	1.37
7	27.31	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	$C_{15}H_{24}O_2$	236	1.51
8	31.27	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	$C_{22}H_{32}O_2$	328	6.45
9	32.21	Diazoprogesterone	$C_{21}H_{30}N_4$	338	2.18
10	35.17	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E)-	$C_{20}H_{34}O$	290	4.61

3.3.2 Compounds Identified*: Cassia Fistula*

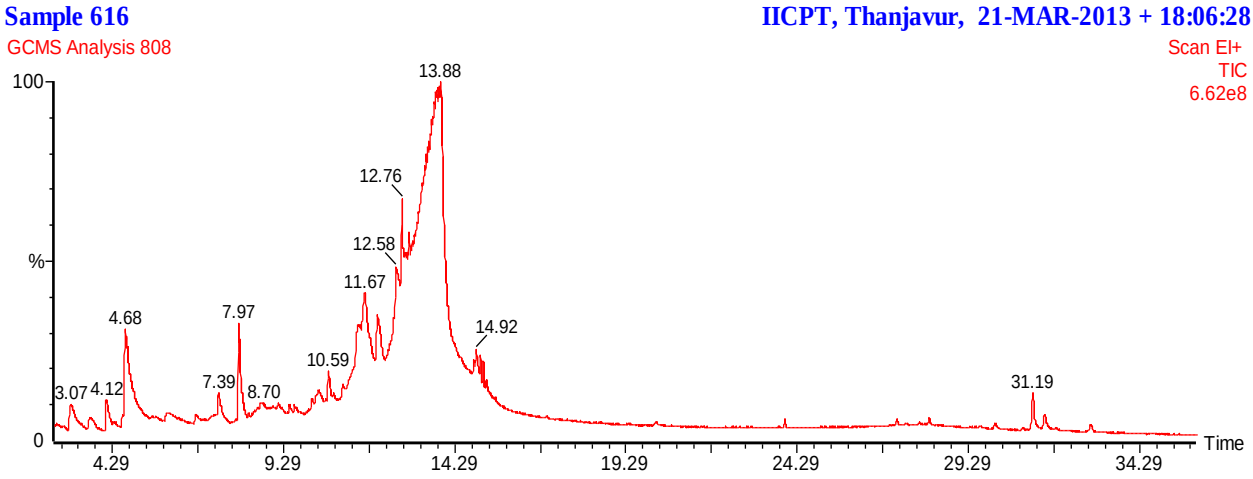

Fig 5: GC-MS chromatogram – *Cassia fistula*

GC-MS chromatogram analysis of the ethanolic extract of *Cassia fistula* showed[Fig 5] sixteen peaks which indicating the presence of sixteen phytochemical constituents. On comparison of the mass spectra of the constituents with the help ofDr Duke's Phytochemical and Ethanobotanical databases, the sixteen phytocompounds were characterized and identified. The various phytochemicals which contribute to the medicinal activities of the plant were shown. The mass spectra of all the phytochemicals identified in the whole plant ethanolic extract of *Cassia fistula*were shown. Of the sixteen compounds identified, the most prevailing compounds were 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, 5-Acetoxymethyl-2-furaldehyde, Oleic Acid, Cholesta-4,6-dien-3-ol, (3b')-, Vitamin E, b'-Sitosterol, Cholest-5-en-3-ol, 24-propylidene-, (3b')-. Among the compounds, four compounds were reported to have anti-microbial property Butanoic acid, 2-methyl-, 2-methylpropyl ester, Pentanoic acid, 1,1-dimethylethyl ester, Valeric acid, 4-tridecyl ester, 2,4;3,5-Dimethylene-l-iditol of the sample showed in [Table 6].

Table 6: Components identified in the sample – *Cassia fistula* [GC- MS study]

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	3.07	Thymine	$C_5H_6N_2O_2$	126	1.16
2	3.65	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	0.49
3	4.12	Butanoic acid, 2-methyl-, 2-methylpropyl ester	$C_9H_{18}O_2$	158	0.77
4	4.68	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	5.28
5	7.39	Pentanoic acid, 1,1-dimethylethyl ester	$C_9H_{18}O_2$	158	1.28
6	7.97	5-Acetoxymethyl-2-furaldehyde	$C_8H_8O_4$	168	1.80
7	10.59	Valeric acid, 4-tridecyl ester	$C_{18}H_{36}O_2$	284	0.97
8	11.67	2,4;3,5-Dimethylene-l-iditol	$C_8H_{14}O_6$	206	12.76
9	12.76	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.35
10	13.88	Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	194	64.82
11	14.92	Oleic Acid	$C_{18}H_{34}O_2$	282	1.50
12	23.94	Squalene	$C_{30}H_{50}$	410	0.08
13	27.21	Cholesta-4,6-dien-3-ol, (3á)-	$C_{27}H_{44}O$	384	0.27
14	28.15	Vitamin E	$C_{29}H_{50}O_2$	430	0.27
15	31.19	á-Sitosterol	$C_{29}H_{50}O$	414	0.75
16	31.53	Cholest-5-en-3-ol, 24-propylidene-, (3á)-	$C_{30}H_{50}O$	426	0.47

4. Discussion

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects[23], whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents[11]. The potential effect of the ethanolic extract of *Cassia fistula* and *Luffaacutangula* was investigated.

Cassia fistula and *Luffaacutangula* contains alkaloids, tannins, flavonoids,terpenes, sugars, and glucosides. Tannins are naturally occurring and water soluble phenolic compounds, which precipitate proteins from aqueous media[6]. Tannins have been shown to possess various biological properties related to antioxidant[4], antinociceptive[7], and anti-inflammatory[5]. A review on tannins and human health has been done by Chung et.al[16]. Condensed tannins of higher molecular weight are commonly called as Phlobatannins[17]. Terpenes are plant secondary metabolites which have antimicrobial activity[8-10]. Flavonoids have activity against antidiabetic and anticancer in human[12,13].

*Cassia fistula*shows the presence of glycoside a natural product, which is used to enhance the cardiac contractile force in patient with congestive heart failure[14] glycoside also plays major role in the cancer therapy[15.]

The plant studied showed the presence of steroids wide used groups of drugs in anaesthetic purpose[19.]. They also play major role in controlling topical disease as eczema etc.

Recent studies suggest that the inflammatory tissue damages are due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites by Cross C.E et al.,[18]. In addition to this, nitric oxide is also implicated in inflammation, cancer, and other pathological conditions[20]. Interactions between superoxide and nitric oxide regulate the vascular tone or inflammation by Conner E.M et al., 1996[21]. The mechanism of targeting reactive oxygen species and prostaglandins involved in the late phase of acute inflammation and pain perception[24].

Using the results obtained from GC-MS, we found the presence of compounds which are active against inflammation.

5. Conclusion

In present study we carried out several phytochemical tests to evaluate the anti-inflammatory activity of *Cassia fistula* and *Luffaacutangula*. Qualitative and Quantitative phytochemical analysis were done. From the results we found that our plant species contains many effective compounds like flavonoids, alkaloids, tannin, anthroquinone etc. Further we analysed our samples using gas chromatography and mass spectrometry (GC-MS).Based on the GC-MS results obtained we conclude that *Cassia fistula* and *Luffaacutangula* have anti-inflammatory activity and on comparison *Cassia fistula* has more.

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6. References

1. Mishra N, Behal KK. Antimicrobial activity of some species against selected microbes.Int J Pharm Sci. 2010;2:187-196.
2. Udayaprakash NK, Bhuvaneswari S, Jahnavi B, Abhinaya K, Rajalin AG, et al. A study on antimicrobial activity of common weeds in Northern District of Tamil Nadu, India. Res J Med Plant.2012;6:341-345.
3. Ajayi IA, Ajibade O, Oderine RA. Preliminary phytochemical analysis screening of some plant seeds. Res J Chem Sci. 2011;1:58-62.
4. KoleckarV, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L, Opletal 331 L Condensed and hydrolysable tannins as antioxidant influencing the health. Mini Rev Med Chem, 2008;8:436-447.
5. Ukoda PO, Cemaluk EAC, Nnamdi OL, Madus EP. Tannins and other phytochemicals of the Samanaeasaman pods and their antimicrobial activities.Afr J Pure Applied Chem. 2011;5:237-244.
6. Martin JS, Martin MM, Tannin assays in ecological studies: Lack of correlation between phenolics, proanthocyanidins and protein-precipitating constituents in mature foliage of six oak species. Oecologia. 1982;54:205-211.
7. 7.S. S. Huang, C. S. Chiu, and H. J. Chen, "Antinociceptive activities and the mechanisms of anti-inflammation of asiatic acid in mice,"*Evidence-Based Complementary and Alternative Medicine*pages, 2011.
8. Andrews RE, Parks LW, Spence KD. Some effects of Douglas fir terpenes on certain microorganisms. App. Environ. Micronbiol. 1980;40:301-304.
9. Islam AK, Ali MA, Sayeed A, Salam SM, Islam A, Rahman M, Khan GR, Khatun S. An antimicrobial terpenoid from Caesalpiniapulcherrima Swartz.Its characterization, antimicrobial and cytotoxic activities. Asian J. Plant Sci. 2003;2:17-24.
10. Prabuseenivasan S, JayakumarM,Ignacimuthu S. In vitro antibacterial activity of some plant essential oils.BMC Complement.Altern. Med. 2006;6:39.
11. GovindarajanM.Bioefficacy of *Cassiafistula*Linn. (Leguminosae)l eaf extract against chilnm gnnya vector,*Aedesaegypti*(Diptera: Culicidae).Eur.Rev. Med.Phannacol.Sci. 2009;13:99-103.
12. Chahar MK, sharma N. Dobhal MP, Joshi YC, Flavonoids: A versatile source of anticancer drugs. Pharmacogn Rev.2011;5:1-12.
13. Ghasemzadeh A, Gasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. J. Med. Plants Res.2011;5:6697-6703.
14. Evans WC, Tease and Evans' Pharmacognosy 14thEdn. W.B..Saunders Company, London; 1996.
15. Winnicka K, Bielawski K, Bielawski A. Cardiac glycosides in cancer research and cancer therapy. ActaPoloniaePharmaceutica, 2006;63:109-115.
16. Chung KT, Wong TY, WeiCl, Huang YW, Lin Y, Tannins and human health: A review Crit Rev Food Sci. Nutri. 1998;38:421-64.
17. Foo LY, Karchesy JJ.Chemistry and Significance of Condensed Tannins, Plenum Press; 1989.
18. Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, McCord, Harman D. Oxygen radicals and human disease. 1987;107(4): 526-46.
19. Shaikh S, Verma H, Yadav N, Jauhari M, Bullangowda J. Applications of Steroid in Clinical Practice: A Review. ISRN Anesthesiology, 2012,doi:10.5402/2012/985495.

20. S. PerwezHussain, Peijun He, Glewood E. Trevers et al., Nitric Oxide is a key Component in Inflammation-Accelerated Tumorigenesis.The Jour of Can Research. 2008;68(17):7130-6.
21. Elaine M.Cooner, Stephen J. Brand, Jonathan M Davis, David Y Kang,Dr. Matthew B. Grisham. Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease: Toxins, mediators, and modulator of gene expression. Official J of the Crohn's and Colitis F of Ame, Inc. 1996;2(2):133-147.
22. www.medicinehunter.com/ethanobotanical-database.
23. S.M.R Gurney et al., Pharmacology, Toxicology and Adverse Effects of Synthetic Cannabinoid Drugs; Forensic Science Review.2014;26-53.
24. V Lakshmi Prasanna et al., Antinociceptive and Anti-Inflammatory activity of TecomaStans leaf extracts. IJRPB.2013;1(2):156-160.
25. Vashista, P.C., 1974. Taxonomy of Angiosperms.P.B.M. Press, New Delhi, India.Balunas.M.J.andA.D. Kinghorn, 2005. Drug discovery frommedicinalplants.LifeSci.•78:431-441.
26. Lee 2003, Medicinal Plants, a powerful health aid. Pp 45-174, Ogunti, E.O, and Elujoba, A.A (1993), Laxative activity of *Cassia fistula*, *Journal of Fitoterapia*. Vol. 64 (5); p: 437-439.
27. Choudhury, B., 1967. Vegetables.National Book Trust, New Delhi, India.Pandey, B.P., 1969. Taxonomy of Angiosperms.S.Chand and Company Ltd. New Delhi, India.,
28. Rahman, A.H.M.M., 2004. Taxonomic Studies of the Cucurbits Grown in the Northern Parts of Bangladesh.M.Phil. Thesis, Department of Botany, University of Rajshahi, Bangladesh.,
29. Vashista, P.C., 1974. Taxonomy of Angiosperms.P.B.M. Press, New Delhi, India.Balunas.M.J.andA.D. Kinghorn, 2005. Drug discovery frommedicinalplants.LifeSci.•78:431-441.
30. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Prospect. 2001;109:69-75.

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