Research Article

Qualitative and quantitative phytochemical analysis of *Cassia hirsuta* seeds

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Abstract

Objective: The present study aim was to analyze quantitatively and qualitatively the phytochemicals as well as the investigating the physicochemical properties from seeds of *Cassia hirsuta* and its chloroform and ethanol extracts. **Materials and Methods:** In this the physico-chemical evaluation, ash values viz., total ash, acid insoluble ash and water soluble ash, sulphated ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive and chloroform soluble extractive value, and loss on drying were determined. Preliminary phytochemical screening where done for presence of carbohydrates, proteins and amino acids, glycosides, alkaloids, phytosteroids, flavonoids, saponins and tannins and phenolic compounds, and total phenolic content was estimated by Folin Ciocalteu's method and total flavonoid content was measured with the aluminium chloride colorimetric assay. **Results:** In results it was found that the seed contains various phytochemicals were present in its ethanol and chloroform extracts. The total phenolic content in the ethanol and chloroform extracts were found 13.7±0.4187 and 7.367±0.2987 mg of gallic acid equivalent weight/g of extract respectively and the concentration of flavonoids in plant ethanol and chloroform extracts of *Cassia hirsuta* were found 114.6±13.33 and 99.56±11.6 mg of quercetin equivalent weight/ g of extract respectively. **Conclusion:** The present study concludes that the seeds of *Cassia hirsuta* have the potential to act as a source of useful drugs because of presence of various phytochemical constituents and high concentration of phenolic and flavonoid compounds.

***Keywords*:** *Cassia hirsuta,* ash values, flavonoids, phenols, phytochemicals

Introduction

The World Health Organization (WHO) estimates that approximately 80 percent of the world's population relies primarily on traditional medicines as sources for their primary health care. Plant-derived products have dominated the human pharmacopoeia for thousands of years almost unchallenged (Alves et al., 2000). In recent years, numerous scientific investigations of traditional herbal remedies for several diseases have been carried out and this investigations shown pathway for the development of alternative drug and therapeutic strategies (Correa et al., 1984). Since the utilization of medicinal plants is increasing, it is interesting to use these plants as a supplement in food taking into account that these plants can present a significant amount of trace elements and other nutrients (Andrade et al., 2005a; Andrade et al., 2005b).

Nowadays using the most modern methods, detailed qualitative and quantitative analysis, isolation and testing of even traces of substances present in plant tissue is possible (Harvey, 2008). Apart from this the extracts of plants have shown interesting area of application as inhibiting the growth and reduction in the number of serious pathogens of disases (Kuete et al., 2006; Kotzekidou et al., 2008), and a great deal of efforts is focused on using available experimental techniques to identify natural antioxidants from plants. In the search for sources of natural antioxidants, in recent years some medicinal plants have been extensively studied for their antioxidant activity and radical scavenging activity (Schinella et al., 2002; VanderJagt et al., 2002). Also, a number of clinical trials have shown that various secondary

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metabolites of plants can be used in the treatment of different cancer types (Butler, 2004).

Cassia hirsuta plant (Holm et al., 1979; Irwin and Barneby, 1982) belongs to the family Caesalpiniaceae and commonly called as stinking cassia and hairy senna (Figure 1).

Figure 1. *Cassia hirsuta* Linn.

It is a terrestrial perennial, erect shrub up to 150 cm in tall, stem rounded, solid glabrons, flowering period from September to December and fruiting in November to January. It is a native of tropical America and now distributed in Malaysia, Indo-China, Thailand, Asian and African tropics, Laos, Java, Brazil, California, New Mexico and India (Holm et al., 1979). In India widely available in Deccan in the Babubudaii Hills of Mysore, Rammdrug, Bellary, also in the Camatic near Madras. Plant is softly tomentose. Branches groved, leaves 15-20 cm long with a gland at base of petiole; Stipules leaner; leaves 4-6 pairs, ovate elliptic, acuminate, rounded or cuneate at base, 10X4 cm. Corimas in penicles, terminal; bract lanceolate, acuminate. Stamens 6-7 antheriferous, 2 larger. Many seeded 14X0.5 cm (Vellingiri et al., 2011).

The leaves are used medicinally for treating herpes. A decoction of leaves is used against irritation of skin in Thailand. In Laos, the seeds are used as a substitute for coffee. Phytomedicinally, the plant parts or extracts are used for treating illness in man. Plants as gifts of nature have many therapeutic properties combined with much nutritive value, which have made their use in chemotherapy as valuable as the synthetic drugs. Herbal organ of the body are used to feed and restore to health those parts, which have become weakened. It is a medicinal plant widely used for stomach troubles, dysentery, abscesses, rheumatism, fever and other diseases. Seeds contain phytotoxin, tannins and 0.25% chrysarobin. Seeds also contain a water soluble sugars extract as D-galactose and D-mannose and medicine for Parkinson's disease. It is also useful in dental caries: powdered seeds are used to massage on gums and teeth (Brenan, 1967; Revathi and Parimelazhagan, 2010; Singh et al., 2007; Jamir et al., 2010).

Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity. During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather, a few new drugs. Plants have successfully passed the tests of commercial screenings.

Preliminary phytochemical screening of plants is also necessary for the discovery and development of novel therapeutic agents with improved efficacy (Ramakrishna et al., 2000; Yadav et al., 2014). The present study aim was to analyze quantitatively and qualitatively the phytochemicals as well as the investigating the physicochemical properties from seeds of *Cassia hirsuta* and its chloroform and ethanol extracts.

Material and methods

Chemicals

Folin-Ciocalteau phenol reagent, gallic acid, quercetine, anhydrous sodium carbonate, methanol, Deionised water, chloroform, benzene, petroleum ether, ethanol, Sodiumnitrite, aluminium tri chloride, sodium hydroxide and all other chemical of laboratory grade.

Plant material

The plant was collected from road side area of Badami road and Raichur road, Bagalkot, Karnataka. It was identified and authenticated and by V G Jadimath, Department of Botany, Government PU College for Girls, Bagalkot-587101, Karnataka.

Preparation of extracts

The seeds were cleaned and air dried then subjected to coarse powdering and passed through a sieve # 44 to get uniform powder size. The collected powder was used for physicochemical analysis and successively extracted with petroleum ether to defat and then by chloroform followed by ethanol for 24 hr by using soxhlet apparatus. After the extraction, solvents was distilled off to get concentrated residue and completely dried by lyophilization and stored in air tight container under refrigeration. Yield of chloroform extract of *Cassia hirsuta* [CECH] was found 1.03% and ethanol extract of *Cassia hirsuta* [EECH] was found 2.52%.

Physicochemical properties of powdered seed of *Cassia hirsuta*

In the physico-chemical evaluation, ash values viz., total ash, acid insoluble ash and water soluble ash, sulphated ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive and chloroform soluble extractive value, and loss on drying were determined (Kokate, 2003). The ash values represent the inorganic salts present in the drug. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of contents of drug.

Determination of total ash value

Three gram of powder of seed *Cassia hirsuta* was taken in a tared silica crucible and incinerated at a temperature not exceeding 450 °C until free from carbon. The resultant ash was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

Acid insoluble ash value

The total ash obtained from 3g of root powder was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash value

The total ash obtained from 3g of root powder was boiled for 5 minutes with 25 ml of water and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Determination of sulphated ash value

The total ash obtained from 3g of root powder was moistened with 1 ml of concentrated sulphuric acid, heated gently until the white fumes were no longer evolved, ignited and weighed. The percentage of sulphated ash was calculated with reference to the air dried drug.

Determination of alcohol soluble extractive value

Accurately weighed powder (5 g) of seed *Cassia hirsuta* was taken and macerated with 100 ml of 95% alcohol for 24 h in a air tight container. The contents were frequently shaken during the first 6 h and allowed to remain for 18 h. After 24 h, the extract was filtered and filtrate was evaporated finally the extract was dried at 105°C to a constant weight and extractible value was calculated as % (w/w) with reference to air dried drug.

Determination of water soluble extractive value

Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform water was used for maceration.

Determination of chloroform soluble extractive value

Chloroform soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform was used for maceration.

Loss on drying

LOD is the loss in weight in % (w/w) resulting from water and volatile matter of any kind that can be driven off under specified conditions. Weigh accurately about 1.5 gm of the powdered drug in a tared porcelein dish and dried at 105 ºC in hot air oven to get constant weight and then weighed. From the difference in weight, the percentage loss on drying with reference to the air-dried substance was calculated.

Qualitative phytochemical analysis of *Cassia hirsute* extracts

One gram of the chloroform and ethanol extracts of *Cassia hirsuta* were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration 1% (w/v). The extracts thus obtained were subjected to preliminary phytochemical screening following the standard procedure (Harborne, 1998; Kokate, 2003).

Tests for Carbohydrates

Molish's test

To 2-3 ml aqueous extract, added few drops of a-naphthol solution in alcohol, shaken and added concentrated H2SO4 from sides of the test tube was observed for violet ring at the junction of two liquids.

Fehling's test

1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min was observed for a yellow, then brick red precipitate.

Tests for Proteins and Amino acids

Biuret test

To 3 ml test solution added 4% NaOH and few drops of 1% CuSO4 solution observed for violet or pink colour.

Million's test

Mixed 3 ml test solution with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red color was observed.

Ninhydrin test

The 3 ml test solution and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. observed for purple or bluish color.

Tests for Glycosides

Hydrolysis of extract

A minimum quantity of the extracts is hydrolyzed with hydrochloric acid for few minutes on water bath and the hydrolysate is subjected to the following tests.

Legal's test

To the hydrolysate 1 ml of the pyridine and few drops of sodium nitroprusside solution added, then it is made alkaline with sodium hydroxide solution. Color change shows the presence of glycosides.

Borntrager's test

Hydrolysate is treated with chloroform and the chloroform layer is separated. To this, equal quantity of dilute ammonia solution is added. Color changes in the ammonical layer shows the presence of glycoside.

Baljet's test

A test solution observed for yellow to orange color with sodium picrate.

Tests for Alkaloids

Mayer's test

To the 1 ml of extract, add 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.

Dragendroff's test

To 1 ml of the extract, add 1 ml of Dragendroff's reagent (potassium bismuth iodide solution). An orange red precipitate indicates the presence of alkaloids.

Hager's test

To 1 ml of the extract, add 1 ml of Hager's reagent (saturated aqueous solution of picric acid). A yellow colored precipitate indicates the presence of alkaloids.

Wagner's test

To 1 ml of the extract, add 1 ml of Wagner's reagent (iodine in potassium iodide solution). Formation of reddish brown precipitate indicates the presence of alkaloids.

Tests for Phyto Steroids

Small quantity of extract is dissolved in 5 ml of chloroform separately. The above obtained chloroform solutions are subjected to Salkowski and liebermann- Burchard tests.

Salkowski test

To the 1 ml of above prepared chloroform solution few drops of concentrated sulphuric acid is added. Formation of brown ring indicates the presence of phytosterols.

Liebermann-Burchard test

The above prepared chloroform solutions are treated with few drops of concentrated sulphuric acid followed by 1 ml of acetic anhydride solution. A bluish green color solution shows the presence of phytosterols.

Tests for Flavonoids

Shinoda test

To dried powder or extract added 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink color was observed.

Ferric Chloride test

Test solution with few drops of ferric chloride solution shows intense green color. **Alkaline reagent test**

Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow color, which becomes colorless on addition of few drops of dilute acid.

Lead Acetate solution test

Test solution with few drops of lead acetate solution (10%) gives yellow precipitates.

Tests for Saponins

Foam test

The drug extract or dry powder was shaken vigorously with water. Persistent foam was observed.

Tests for Tannins and Phenolic Compounds

Ferric chloride test

To 2-3 ml of extract, add few drops of 5% FeCl3 solution a deep blue-black color was observed.

Lead acetate solution test

To 2-3 ml of extract, add few drops of lead acetate solution a white precipitate were observed.

Dilute HNO_3 test

To 2-3 ml of extract, add few drops of Dilute HNO_3 solution, a reddish to yellow color was observed.

Quantitative phytochemical analysis of chloroform and ethanol extract of *Cassia hirsuta*

Determination of total phenol content

Total phenolic content was estimated by Folin Ciocalteu's method. 1 ml of aliquots and standard gallic acid (12.5, 25, 50, 100 and 200 µg/ml) was positioned into the test tubes and 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent was mixed and shaken. After 5 minutes, 1.5 ml of 20 % sodium carbonate was added and volume made up to 10 ml with distilled water. It was allowed to incubate for 2 hours at room temperature. Intense blue color was developed. After incubation, absorbance was measured at

750 nm by using UV-visible spectrophotometer. The extracts were performed in triplicates. The blank was performed using reagent blank with solvent. Gallic acid was used as standard. The calibration curve was plotted using standard gallic acid. The data for total phenolic contents of extrcts were expressed as mg of gallic acid equivalent weight (GAE)/ 1 g of extract (Bhalodia et al., 2011; Patel and Patel, 2010).

Determination of total flavonoid content

Total flavonoid content was measured with the aluminium chloride colorimetric assay. 1ml of aliquots and 1ml standard quercetin solution (50, 100, 200 and 400 µg/ml) was positioned into test tubes and 4ml of distilled water and 0.3 ml of 5 % sodium nitrite solution was added into each. After 5 minutes, 0.3 ml of 10 % aluminum chloride was added. At 6th minute, 2 ml of 1 M sodium hydroxide was added. Finally, volume was making up to 10 ml with distilled water and mix well. Orange yellowish color was developed. The absorbance was measured at 510 nm by using UV-visible spectrophotometer. The blank was performed using distilled water. Quercetin was used as standard. The samples were performed in triplicates. The calibration curve was plotted using standard quercetin. The data of total flavonoids of extracts were expressed as mg of quercetin equivalents/ 1 g of extract (Pallab et al., 2013; Patel et al., 2012; Sathishkumar et al., 2008).

Results

Physicochemical properties of seed *Cassia hirsuta*

The ash values viz., total ash, acid insoluble ash, water soluble ash and sulphated ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive and chloroform soluble extractive values, loss on drying were calculated and recorded. Results were summarized in table 1.

Table 1. Physicochemical properties of seeds of *Cassia hirsute*

Parameters	Values in %(w/w)
Ash Value	
Total ash value	3.52
Acid insoluble ash value	0.4167
Water soluble ash value	1.403
Sulphated ash	3.42
Extractive value	
Alcohol soluble extractive value	6.2
Water soluble extractive value	21.8
Chloroform soluble extractive value	4.4
Loss on drying	4.247

Preliminary phytochemical studies

Preliminary phytochemical screening to detect the different chemical principles present in chloroform extracts viz., carbohydrates, proteins, amino acids, glycosides, alkaloids and flavonoids were present and in ethanol extract carbohydrates, proteins, amino acids, glycosides, alkaloids, steroids, flavonoids, tannins and phenolic compounds were present. Results were summarized in table 2.

Total phenolic contents

The total phenolic contents in the examined ethanol and chloroform extracts were found 13.7±0.4187 and 7.367±0.2987 mg of gallic acid equivalent weight/ g of extract respectively. The highest concentration of phenols was measured in ethanol extract and chloroform extracts contains considerably smaller concentration of phenols. The total phenolic contents in plant extracts of the species *Cassia hirsuta* depends on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction. Results were summarized in table 3.

Table 2. Qualitative phytochemical analysis of chloroform and ethanol extract of *Cassia hirsuta*.

Phytochemical constituents	Name of the test	CECH	EECH
Carbohydrates	Molish's test	Positive	Positive
	Fehling's test	Positive	Positive
Proteins and Amino acids	Biuret test	Positive	Positive
	Million's test	Positive	Positive
	Ninhydrin test	Positive	Positive
Glycosides	Legal's test	Positive	Positive
	Baljet's test	Negative	Positive
Alkaloids	Mayer's test	Negative	Positive
	Dragendroff's test	Positive	Positive
	Hager's test	Negative	Positive
	Wagner's test	Positive	Positive
Phyto Steroids	Salkowski test	Negative	Positive
	Liebermann-Burchard test	Negative	Negative
Flavonoids	Shinoda test	Negative	Positive
	Ferric Chloride test	Positive	Positive
	Alkaline reagent test	Positive	Negative
	Lead Acetate solution test	Negative	Positive
Saponins	Foam test	Positive	Negative
Tannins and Phenolic Compounds	5% $FeCl_3$ solution	Positive	Positive
	Lead acetate solution	Negative	Positive
	Dilute HNO_3	Negative	Positive

CECH=Chloroform extract of *Cassia hirsuta* and EECH=Ethanol extract of *Cassia hirsuta*

Table 3. Total phenolic content of chloroform and ethanol extract of *Cassia hirsuta*

Extracts	Phenolic content (mg of gallic acid equivalent weight/g of extract)
CECH	7.367±0.2987
EECH	13.7±0.4187

All values are expressed in Mean±SEM. CECH=Chloroform extract of *Cassia hirsuta* and EECH=Ethanol extract of *Cassia hirsuta*

Table 4. Total flavonoid content of chloroform and ethanol extract of *Cassia hirsuta*

Extracts	Flavonoid content (mg of quercetin equivalent weight/ g of extract)
CECH	99.56±11.6
EECH	114.6±13.33

All values are expressed in Mean±SEM. CECH=Chloroform extract of *Cassia hirsuta* and EECH=Ethanol extract of *Cassia hirsuta*

Total flavonoid content

The concentration of flavonoids in plant ethanol and chloroform extracts *Cassia hirsuta* were found 114.6±13.33 and 99.56±11.6 (mg of quercetin equivalent weight/ g of extract) respectively. Ethanol extracts contains the highest flavonoid concentration and low flavonoid concentration was measured in chloroform extract. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation. Results were summarized in table 4.

Discussion

As the plant *Cassia hirsuta* is used in the traditional medicine for the treatment of some ailment, it is very essential to standardize it for its use as a drug. The quantitative determinations of some phamacognostic parameters are useful for setting standards for crude drugs. The detection of adulteration or errors in handling of the drug depends upon the evaluation of important parameters like physical constants. The purity of the drug i.e. the presence or absence of foreign inorganic matter can be indicated by the various ash values.

Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards. The total ash, acid insoluble ash, water soluble ash and sulphated ash was found to be 3.52%, 0.4167%, 1.403% and 3.42%. This percentage clearly indicates that the root is best for drug action and effects. The Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage. The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value. The water soluble extractive value proved to be higher than alcohol soluble extractive value. It was found to be 21.80%. This shows that the constituents of the drug are more extracted and soluble in water as compared to alcohol. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs. The moisture content of the crude drug was found below 4.247%.

Phytochemical analysis is of paramount importance in identifying new source of therapeutically and industrially valuable compounds having medicinal plants have been chemically investigated (Ambasta et al., 1986).

The present study was carried out on the *Cassia hirsuta* to standardize its components, it revealed the presence of different active phytochemical constituents and these constituents were qualitatively and quantitatively analyzed using different analytical and spectroscopic methods. The phytochemical screening of seeds of *Cassia hirsuta* and CECH and EECH showed that this contains; carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, flavonoids, tannins and phenolic compounds are present in ethanol extract and apart from steroids all other constituents also present in chloroform extract. The variations in phytochemical contents of the plant are due to number of environmental factors such as climate, altitude, rainfall etc. (Kokate et al., 2004). These variations of phytochemical constituents of the plant seemed to be the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that have vital role for good health (Bhumi and Savithramma, 2014).

Phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoids, including flavones, flavanols and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity in vitro and also act as antioxidants in vivo (Soobrattee et al., 2005; Geetha et al., 2003; Shimoi et al., 1996).

Conclusion

In the present study it was concluded that the seeds of *Cassia hirsuta* have the potential to act as a source of useful drugs because of presence of various phytochemical constituents such as alkaloids, flavonoids, phenol,

terpenoids, saponin and carbohydrates. These phytoconstituents seemed to be the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital role for good health. With this it was also found high contents of phenolic and flavonoid compounds indicated that these compounds contribute to the antioxidant activity.

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Conflicts of interest: Not declared.

References

Alves TM, Silva AF, Brandão M, Grandi TS, Smânia E, Smânia Júnior A, Zani CL. 2000. Biological screening of Brazillian medicinal plants. Memórias do Instituto Oswaldo Cruz, 95(3):367-373.

Ambasta SP, Ramachandran K, Kashyapa K, Chand R. 1986. Useful plants of India. Publication and information directorate. Council of Scientific and Industrial Research, New Delhi 433–7.

Andrade ECB, Alves SP, Takase I. 2005a. Avaliação do uso de ervas medicinais como suplemento nutricional de ferro, cobre e zinco1. Ciencia e Technologia de Alimentos, 25(3):591-596.

Andrade ECB, Alves SP, Takase I. 2005b. Extração seqüencial de cobre, ferro e zinco em ervas medicinais. Ciencia e Technologia de Alimentos, 25(4):844-848.

Bhalodia N, Nariya P, Acharya R, Shukla V. 2011. Evaluation of *in vitro* antioxidant activity of flowers of *Cassia fistula* Linn. International Journal PharmTech, Research, 3(1):589-599.

Bhumi G, Savithramma N. 2014. Screening of pivotal medicinal plants for qualitative and quantitative phytochemical constituents. International Journal of Pharmacy and Pharmaceutical Sciences, 6(3):63–65.

Brenan JPM. Leguminosae, subfamily Caesalpinioideae. In: Milne-Redhead E, Polhill RM. 1967. Flora of Tropical East Africa. Crown Agents for oversea governments and administrations: London, UK, 230.

Butler MS. 2004. The role of natural product chemistry in drug discovery. Journal of Natural Products, 67:2141-53.

Correa MP. 1984. Dicionario das plantas uteis do Brasil e das exoticas cultivadas. Rio de Janerio: Instituto Brasileiro de Desenvolvimento Florestal.

Geetha S, Sai Ram M, Mongia SS, Singh V, Ilavazhagan G, Sawhney RC. 2003. Evaluation of antioxidant activity of leaf extract of sea buckthorn (*Hippophae rhamnoides* L.) on chromium (VI) induced oxidative stress in albino rats. Journal of Ethnopharmacology, 87:247–251.

Harborne JB. 1998. Phytochemical methods. A guide to modern techniques of plant analysis. 3rd Edn., Chapman and Hall Int. Ed., New York.

Harvey AL. 2008. Natural products in drug discovery. Drug Discovery Today, 13:894-901.

Holm LG, Pancho JV, Herberger JP, Plucknett DL. 1979. A Geo- graphical Atlas of World Weeds. Knieger Publishing Company: Florida.

Irwin HS, Barneby RC. 1982. The American Cassiinae, A Synoptical revision of Leguminosae tribe Cassieae subtribc Cassiinae in the New World. Memoirs of The New York Botanical Garden, 35:435.

Jamir NS, Takatemjen L. 2010. Traditional knowledge of Lotha-Naga tribes in Wokha district, Nagaland. Indian Journal of Traditional Knowledge, 9(1):45-8.

Kokate CK. 2003. Practical Pharmacognosy. 4th Edn, Vallabh Prakashan, New Delhi 122-126.

Kokate CK, Purohit AP, Gokhale. 2004. Pharmacognosy, second ed. Vallabh Prakashan, New Delhi, pp. 466-470.

Kotzekidou P, Giannakidis P, Boulamatsis A. 2008. Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate. LWT- Food Science and Technology, 41:119-197.

Kuete V, Nguemeving JR, Beng VP, Azebaze AGB, Etoa FX, Meyer M, Bodo B, Nkengfack AE. 2006. Antimicrobial activity of the methanolic extracts and compounds from Vismia laurentii De Wild (Guttiferae). Journal of Ethnopharmacology, 109:372-379.

Patel A, Patel NM. 2010. Estimation of flavonoid, polyphenolic content and *in vitro* antioxidant capacity of leaves of *Tephrosia purpurea* Linn. (Leguminosae). International Journal of Pharmaceutical Sciences and Research, 1(1):66-77.

Patel S, Patel J, Patel RK. 2012. To study proximate analysis & biological evaluation of *Triphala Guggulu* formulation. International Journal PharmTech, Research, 4(4):1520-1526.

Pallab K, Tapan B, Tapas P, Ramen K. 2013. Estimation of total flavonoids content (TPC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. Journal of Drug Delivery and Therapeutics, 3(4):33-37.

Ramakrishna S, Ramana KV, Mihira V, Kumar BP. 2000. Evaluation of anti-inflammatory and analgesic activities

of *Solanum trilobatum* Linn. Roots. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2(1):701-5.

Revathi P, Parimelazhagan T. 2010. Traditional knowledge on medicinal plants used by the Irula Tribe of Hasanur Hills, Erode district, Tamil Nadu, India. Ethnobotanical Leaflets, 14:136-60.

Sathishkumar T, Baskar R, Shanmugam S, Rajasekaran P, Sadasivam S, Manikandan V. 2008. Optimization of flavonoids extraction from the leaves of Tabernaemontana Heyneana Wall. Using L16 orthodesign. Nature and Science, 6(3):10-21.

Singh J, Kumar A, Budhiraja S, Hooda A. 2007. Ethnomedicine: use in dental caries. Brazilian Journal of Oral Sciences, 6(21):1308-12.

Schinella GR, Tournier HA, Prieto JM, Mordujovich de Buschiazzo P, Rios JL. 2002. Antioxidant activity of anti-inflammatory plant extracts. Life Sciences, 70:1023-33.

Shimoi K, Masuda S, Shen B, Furugori M, Kinze N. 1996. Radioprotective effects of antioxidative plant flavonoids in mice. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 350:153–161.

Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun OT. 2005. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 579:200–213.

Vellingiri V, Aruna N, Hans KB. 2011. Antioxidant Potential and Health Relevant Functionality of Traditionally Processed *Cassia hirsuta* L. Seeds: An Indian Underutilized Food Legume. Plant Foods for Human Nutrition,66:245–253.

VanderJagt TJ, Ghattas R, Vanderjagt DJ, Crossey M, Glew RH. 2002. Comparison of the total antioxidant content of 30 widely used medicinal plants of New Mexico. Life Sciences, 70:1035-40.

Yadav M, Chatterji S, Gupta SK, Watal G. 2014. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. International Journal of Pharmacy and Pharmaceutical Sciences, 6(5):539-42.