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STUDY OF THE TRADITIONAL MEDICINAL POTENTIAL OF WILD CASSIA TORA L. LEAVES COLLECT FROM WESTERN GHATS

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Abstract

Cassia tora L. a small, annual weed and well known herbs used in traditional medicine. Traditional they have been used as medicine to cure for various diseases. *C. tora* L. extract was used for cotton and silk fabrics dyeing. The present study examined the quantitative and qualitative study of the major bioactive components of *C. tora* L. leaves. In qualitative screening presence of saponins, flavonoids and absence of tannins were observed. Also the quantitative estimation of protein, carbohydrate, L-Dopa, inorganic phosphate and Vitamin C were carried out. The methanol extract of *C. tora* L. leaves used for antimicrobial activity showed the significant antimicrobial activity against *Salmonella typhi*, *Staphylococus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*. The GC-MS was found that the main constituents of squalene and furan in *C. tora* L. This vegetable is used as a good source of nutrients and it would be an option for other leafy vegetables and an antimicrobial property which may use for the curing disease.

Keywords: *Cassio tora* L., Phytochemical compounds, GC-MS analysis, Antimicrobial activity

1. Introduction

The medicinal plants offer as therapeutic agents in traditional medicines used at the global level for human health. The therapeutic potential of plants has been utilized in traditional medicines such as Ayurveda, Siddha, Chinese and Unani etc. *Cassia tora* L. from family *Caesalpiniaceae*, is annual, dispersed throughout tropical and subtropical zones of the world as a small weed. It is widely distributed in tropical Asian countries. *C. tora* L., grows as a weed in the rainy season, it's an oriental herb native to south Asia, and can grow 1-2 m in height and commonly known as taykala in Maharashtra, India.

The *C. tora* L. medicinal properties described in the ancient ayurvedic literature and well known herbs used in ayuverdic traditional medicine. The plant is widely consumed as a potent source of sennosides (laxative), and enlisted in the World Health Organisation's 'List of Essential Medicines' (WHO, 2014). It's medicinal potentials have been described in the traditional Chinese medicine (TCM) and Ayurvedic practices with the special reference to cure psoriasis and other skin degenerative disorders (Vijayalakshmi and Madhira, 2014). In addition to this, the plant was also used traditionally to cure diabetes, dermatitis, constipation, cough, cold and fever, etc (Meena *et al*., 2010). The plant harbors anti-proliferative, hypolipidemic, immunostimulatory, and anticancerous properties (Abraham *et al*., 2009). *C. tora* possessed a large amount of flavonoids, the potent antioxidants (Pawar and Dmello 2010; Vats and Kamal, 2010). Plant samples from different geographical origins have different biochemical compositions due to variations in the environmental conditions and genetic reasons (Said, *et al*., 2011; Kumar and Roy, 2018).

The *C. tora* L. has conventionally used in the treatment of ringworm (Dutta, 1985), constipation and leprosy (The Wealth of India, CSIR, 1991), jaundice (Gewali, 2008). They also have countless therapeutic potential due to the presence of medicinally active compounds, including anthraquinones, chrysophanol, emodin, chalcone, rhein, basseol, euphol, etc. These compounds have antiprolifrative, hypolipidemic immuno stimulatory, anticancers, antimutagenic and hepatoprotective properties. *C. tora* L. is used to treat diabetes, dermatitis, cough, cold and fever, etc (Jain and Patil, 2010; Meena *et al*., 2010). The roasted or dried seed of *C. tora* L. is used for improving vision in Chinese herbal medicine (Namba, 1980).

C. tora L. have been reported for their valuable role in the form of decoctions, infusions and tinctures in skin diseases like psoriasis, leprosy, etc (Horvath, 1992; Zahra *et al*., 2000; Cordova *et al.*, 2002; Harrison, 2003). *Cassia tora* L. roots has secretes a more amount of citrate in response to aluminum tolerant (Ma *et al*., 1997). In mosquito studies is reported by Kalyanasundaram and Das showed effective results with *C. tora* L. (Kalyanasundaram and Das, 1985). A further identification of citrate synthesis in roots showed that aluminum could stimulate a significant increase in citrate synthase (CS) activity as well as citrate accumulation (Yang *et al*., 2003). *C. tora* L. leaves extract has anthraquinones, they used in dyeing of cotton and silk fabrics (Lee and Kim, 2004). In this study, preliminary was investigated phytochemicals and studied the antimicrobial activity against the pathogenic bacterial strains.

2. Materials and Methods

2.1 Collection of plant material

Fresh leaves of *C.* tora L., free from disease were collected from Chandgad area in district Kolhapur, MH, India. These leaves were generally grown in agricultural field as a weed. The collected leaves were washed carefully in running tap water and then cleaned in distilled water and shaded the dried leaves were crushed using laboratory blender. The fine powder of *C.* tora L. leaves was stored in airtight closed bottles.

2.2 Test microorganisms

The test bacteria *Salmonella typhi* NCIM 5021, *Staphylococus aureus* NCIM 9144, *Escherichia coli* NCIMB 12210 and *Pseudomonas aeruginosa* NCIM 2036 were used for the antimicrobial activity. All bacterial strains were routinely maintained on the nutrient slants containing (g l^{-1}); NaCl 5.0, bacteriological peptone 10.0, yeast extract (2.0), sodium chloride 5.0 and agar 15.0. All the cultures were stored at 4 ^{o}C.

2.3 Preparation of leaf extract

Water and methanol was used for the extraction of phytochemicals from the *C.* tora L. leaves. Appropriate quantity of fresh *C.* tora L. leaves (1:2) was dissolved in water for water extraction. Ten grams of powdered *C.* tora L. leaves was dissolved in 100 ml of methanol in a conical flask, raped aluminum foil for solvent extraction. Both flask kept on rotatory shaker for 2 h at 120 rpm. Then it was sonicated for 15 min for the maximum extraction of phytocompounds in water. Then the sample was filtered with the help of Whatman filter paper. The collected filtrate was then used for phytochemical Analysis (Patil *et al*., 2016).

2.4 Antimicrobial assay

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts (Valgas *et al*., 2007). For agar well method wells (6 mm diameter) were punch into the agar medium and filled with 100 µl (100 mg ml^{-1}) of leaves extract and solvent blanks as control. An incubated antimicrobial activity assay plates for 24 h at 37 °C. After incubation inhibition zone was determined by measuring the diameter of inhibition zone in mm.

2.5 Phytochemical Analysis

The **c**arbohydrates, protein, inorganic phosphate and vitamin C estimation were carried by using 3, 5-Dinitrosalicylic acid (DNSA) , lowery, fiske subbarow and 2,6-Dichlorophenolindophenol (DCPIP) methods respectively described in plummer (Plummer, 1888). The L-Dopa estimation was carried by Arnow's method (Surwase *et al*., 2012).The tests of flavonoid, saponins and tannins were carried out with the aqueous extract of *C.* tora L. by using standard procedure of qualitative phytochemical analysis (Ajayi *et al*., 2011; Patil, 2015). Test for flavonoid *C.* tora L., leaves powder was 1:10 (w/v) diluted with of distilled water and boiled for 5 minutes and filtered. In the 1 ml of filtrate was added few drops of 20 % sodium hydroxide solution. The

yellow color change to colorless after addition of acid solution to confirm the presence of flavonoids in test sample.

Test for tannins use a gram of sample was boiled with distilled water for 5 min and it filtered while hot. The filtrate was added a few drops (2-3) of 10 % ferric chloride. The observation for color change and formation of precipitates were done. A brownish-green or bluish-black precipitate showed the presence of tannins.

Test for saponins use 1 gm of crude sample powder was boiled with 5 ml of distilled water and filtrate was taken the filtrate was added 3 ml distilled water and was shaken continuously for 5 min. If frothing was observed on warming that is an indication of presence of saponins.

2.6 GC-MS Analysis

Metahnol extract of *C. tora* L. was subjected for the GC-MS analysis, for the identification of metabolites. Gas chromatography–mass spectrometry (GC-MS) analysis was performed using a QP2010 gas chromatography coupled with a mass spectrometer (Shimadzu, Japan). The mass spectral analysis was carried out by putting a dye sample in DI (direct injection) mode by keeping ionization voltage at 70 eV and ion source temperature at 220^{o}C. Gas chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m; XTI-5). The initial column temperature was 80^{o}C for 2 min, then increased linearly at 10^{o}C min^{-1} to 280^{o}C and held for 7 min. The temperature of the injection port was 280^{o}C, and the GC/MS interface was maintained at 280^{o}C. Helium was used as the carrier gas at flow rate 1.0 ml min^{-1}. Spectra of unknown component were identified by comparison of retention time, the fragmentation pattern, as well as the mass spectra in the National Institute of Standards and Technology (NIST) spectral library.

3. Result

3.1 Phytochemical analysis

The vegetables have the different bioactive components and they play important role in the treatment and curing diseases. The presence of secondary metabolites is the very important towards the medical research. The phytochemical active compounds of *Cassia tora* L. leaves were quantitatively and qualitatively analyzed.

The qualitatively screening was seemed to be presence of flavonoids, saponins and absence of tannins. On the qualitative investigation confirmed the presence of flavonoids and saponins and they may useful for decreasing the risk of disease.

The quantitatively results are presented in figure 1. The quantitative estimation of protein, carbohydrate, L-Dopa, inorganic phosphate and Vitamin C has carried out. The quantitatively estimation of protein, carbohydrates, L-Dopa, inorganic phosphate and vitamin C were showed 0.033, 0.002, 0.0033, 0.0037 and 0.0133 mg gm^{-1} in concentration respectively. The protein concentration was observed maximum compare to other tested phytochemicals. The present study carried out on the *C. tora* L. leaves revealed the presence of

medicinal active components. Also the medicinally important L-DOPA was present in the *C. tora* L. leaves and they considered as the most potent drug available in the market for the treatment of different disease.

3.2 Antimicrobial activity screening

The results of testing antimicrobial activity of *C. tora* L. leaves methanol extract against four bacterial strains are presented in figure 2. The inhibition zones 16 mm, 5 mm, 10 mm and 9 mm in diameter showed against *Salmonella typhi* NCIM 5021, *Staphylococus aureus* NCIM 9144, *Escherichia coli* NCIMB 12210 and *Pseudomonas aeruginosa* NCIM 2036 respectively. The methanol extract of *Cassia tora* L. leaves showed the significant antimicrobial activity against all the tested pathogens. It is evident that methanol extract showed the maximum (16 mm) against *S. typhi*. The secondary metabolites of *C. tora* L. leaves may be involved in the bacterial growth inhibition.

3.3 GC-MS analysis of Cassia tora L.

GC-MS chromatogram of the methanolic extract of *C. tora* L. leaves showed different peaks which indicating the presence of various phytochemical constituents. The obtained mass spectra of the constituents present in the extract compared with the NIST library. The phytochemical compounds present in the *C. tora* L. leaves with their molecular formula, molecular weight and their significant properties were presented in (Table 1). It was found that the main two constituent's squalene (molecular weight 410) and furan (molecular weight 218) of leaves, which having various medicinal and pharmaceutical activities like antibacterial, antiviral, antioxidant, antitumor and anticancer properties. Too it has antimicrobial activity against pathogens so it may help in some kind of curing disease.

4. Discussion

The leaves of *Cassia tora* L. have the different bioactive components and they play significant role in the treatment and curing diseases. The presence of L-DOPA, saponins and flavonoids in *C. tora* L. is the very important towards the medical research. In the market for the treatment of Parkinson's disease L-DOPA is consider as the most potent drug available (Kofman, 1971; Surwase *et al*., 2012). Also the saponins showed a different biological activity and have useful pharmacological properties such as anti-parasite, anti-inflammation and anti-virus properties (Just *et al*., 1998; Traore *et al*., 2000), anti-cholesterolemic (Oakenfull, 1981). Some evidences demonstration that the saponins can kill tumor cells via different signaling pathways, by triggering death receptors (Cheung *et al*., 2005), targeting mitochondria (Wang *et al*., 2004), and inducing oxidative stress (Kim 1999).

Flavonoids are the major polyphenols present in wide variety of plants and they help in plant defense mechanism against microorganisms, herbivores and insects. These polyphenols derived as nutritional constituents and involved in a number of medicines (Wollenweber, 1988). Flavonoids have extensive variety of biochemical and pharmacological properties including anti-oxidation, anti-inflammation, anti-platelet, anti-

thrombotic action and anti-allergic effects. (Middleton and Kandaswami, 1992; Cooks and Samman, 1996). In view of their pharmacological activities they have a great therapeutic potential.

In the GC-MS analysis squalene and furan was found in the *Cassia tora* L. leaves. The squalene is not susceptible to peroxidation and important role in the protecting human skin surface from lipid peroxidation due to exposure to UV light and other sources of oxidative damage (Kohno *et al*., 1995, Pham *et al*., 2015).The squalene improved the anti-tumor activity and cytotoxicity of adriamycin, 5-fluorouracil, bleomycin and cis-diammine dichloro platinum) and had the anticancer properties (Nakagawa *et al*., 1985). The squalene has a alternate to paraffin that improve the removal of [14C] hexa-chloro-benzene (HCB) and organo chlorine xenobiotic (Richter *et al*., 1982). In recent clinical study squalene from seed oil in the form of microemulsion is use for the treatment of COVID-19 (Ebrahimi *et al*., 2022).

Many 2-arylbenzofuran derivatives are well known to exhibit a broad range of biological activities, including anticancer (Navarro *et al*., 2001), antiproliferative (Ikeda *et al*., 1998), antiviral (Craigo *et al*., 2000), antifungal (Chand *et al*., 2017), antimicrobial activity (Venkateshwarlu *et al*., 2013, Fang *et al*., 2019), antioxidative (Maeda *et al*., 1994), insecticidal (Findlay *et al*., 1997) and cancer preventative activity (den Tonkelaar *et al*., 2001).

The antibacterial activity of *Cassia* tora L. leaves due to the presence of secondary metabolites (flavonoids, saponinsand furan). Recently investigator described the importance of alkaloids, saponins and tannins in several antibiotics, which remain used in treating common pathogenic strains (Kubmarawa *et al*., 2007; Mensah *et al*., 2008). Also the reported flavonoid has antibacterial properties (Tsuchiya *et al*., 1996, Yuan *et al*., 2021). Too it has antimicrobial activity against some pathogens so it will be help in some kind of curing disease. Thus, the presence of phytochemicals may get better the health status of the consumers as a result of the presence of many compounds that play very important for good health.

From the preliminary investigation it was concluded that the plant *C. tora* L. leaves are used as a good source of nutrients and it will be option for another leafy vegetables. Generally to certain extent the consumption of the wild vegetables may contribute to filling the protein gap in human diet at negligible cost. Their consumption could help to reduce malnutrition in people mostly in rural area. There for their cultivation and utilization should be encouraged. So, *C. tora* L. leaves may serve as a new potential source of medicines in the future. The presence of phytochemicals may get better the health status of the consumers as a result of the presence of many compounds that play very important for good health.

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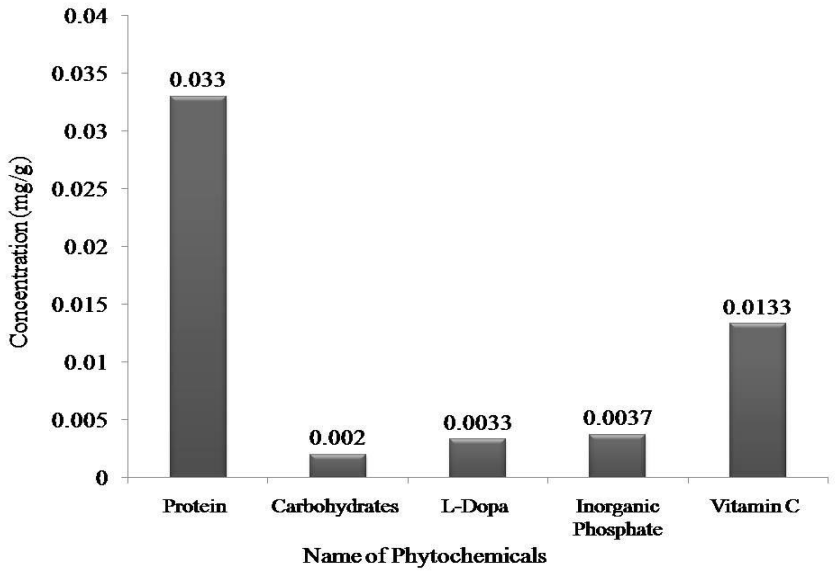

Figure 1. Quantitative estimation of *Cassia tora* L. leaves

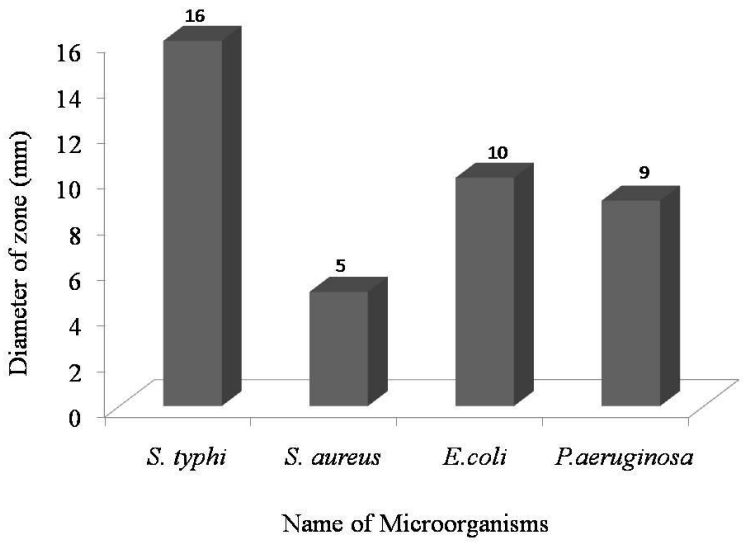

Figure 2. Antibacterial activity of *Cassia tora* L. leaves

Table 1

GCMS analysis of *Cassia tora* L. leaves extract

	Name of component	
	Squalene	**Furan**
Molecular weight	410	264
Molecular formula	$C_{30}H_{50}$	$C_{15}H_{22}O$
Role of compound	Squalene as a Skin and Eye Antioxidant (Kohno, 1995, Huang *et al*., 2009), Antimicrobial activity Yuan *et al*., 2021), Antiviral (Ebrahimi *et al*., 2022)	Antimicrobial activity(Venkateshwarlu *et al*., 2013)