August 30, 2018

PharmacologyOnLine

Archives • 2018 • vol.2 • 227-233

TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID & ANTI-BACTERIAL ACTIVITY OF HYDROALCOHOLIC *ACACIA CATECHU* (L.F.) WILLD ROOTS EXTRACTS FOR TREATMENT OF MOUTH ULCER

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Abstract

There are considerable studies indicating that many herbal extracts have antibacterial effects. The main aim of this study was to determine the in vitro antibacterial activity of hydroalcoholic extract of *Acacia catechu* (L.F.) willd roots against *S. mutans* and *C. albicans*. A thin layer chromatography analysis of hydroalcoholic extract has also been performed and it showed the presence of different types of secondary metabolites. Phytochemical characterization was performed by quantification of total polyphenols, total flavonoids. *Acacia catechu* hydroalcoholic extracts were assayed for antibacterial activity by agar well diffusion method in order to determine the zone of inhibition compared with ofloxacin and fluconazole zone of inhibition as control. In general, the samples were found to be effective against *S. mutans* and *C. albicans*. Hydroalcoholic extract of *Acacia catechu* were effective against *S. mutans* and *C.albicans* at 100 and 50 mg/ml respectively. The results of the present study indicate that hydroalcoholic extract of *Acacia catechu* has an effective and potent antimicrobial activity.

Keywords: *Acacia catechu, Hydroalcoholic extract, Phenolic compounds, Antimicrobial activity*

Introduction

Many plants were used in traditional to treat various diseases. Some of them possess antimicrobial properties. They have been used in different parts of the World to treat human diseases and infections as preservatives and as means of preventing microorganism development [1]. Mouth ulcers are the most prevalent diseases of the oral cavity, most of which, show similar symptoms and may appear with damages of specified limits which are covered by Fibrinoleukocytic membrane [2, 3]. Mouth ulcers may affect the quality of life due to interfere in nutrition, speaking and the social behavior. Mouth ulcers destroy the epithelium which is a natural defensive barrier and therefore, prepare the environment as a proper media for the growth of the microbes and such a matter may cause intensity of the pain. From the faraway times, benefiting from the traditional medicine for decreasing the pains in the human has been effective and the traditional medications are the most useful resource for treatment [4, 5]. *Acacia catechu* (L.F.) willd is a moderate sized tree and belongs to the family Leguminosae. It is 9-12 meter high having dark colored and rough bark with young shoots of dark brown purple shade. It is found in Central India, Northeast Himalayas, Punjab, Bihar and Myanmar (Burma). The bark of *Acacia catechu* is bitter and it was reported to have soothing and astringent activity in bowel and also anti-dysenteric, antidiarrhoea, antipyretic and antihelminthic effects [6]. *Nadkarni KM* (1996) described that extract obtained from bark, wood, flowering tops and gum contains tannic acid 35%, catechuic acid or catechin, catechu red, tannin gum, guercetin and ash. It was used in diarrhea, haemorrhages, bedsores, cracked nipples, gonorrhea, syphilis, leprosy, ulcer and gonorrheal joint pains [7].Considering the high prevalence of oral diseases due to oral pathogens and the recent public interest in medicinal plants, this study aimed to assess the effect of hydroalcoholic extract of *Acacia catechu* on *S. mutans* and *C. albicans in vitro*. Furthermore, phytochemical characterization was performed by quantification of total polyphenols, total flavonoids in the obtained herbal extracts.

Materials and Methods

Collection

The plant material (Root) was purchased from local market of Bhopal. The sample was identified by senior Botanist Dr. Pradeep Tiwari, Doctor Hari Singh Gour Vishwavidyalaya (M.P.) by comparing with the voucher specimen.

Extraction of plant material

100 g. of *Acacia catechu* (L.F.) willd dried roots were exhaustively extracted with hydroalcoholic extract solvent (Ethanol 70%) and using drug-solvent ratios (1:2) using maceration process (10 hrs.). The extracts were evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

Determination of percentage yield

The percentage yields of each extract were calculated by using following formula [8].

Percentage yield= Weight of extract/Weight of powdered drug taken x 100

Qualitative evaluation

Phytochemical tests were done as per the methods given in [9-10].

Chromatographic analysis

Thin layer chromatography (TLC)

The chromatographic screening of the extract was performed by thin layer chromatography (TLC) on silica plates (60F254, aluminum backed, 200 µm layer thickness, 10.0 x 5.0 cm). The presence of secondary metabolite was investigated using adequate development systems and revealers [11-14]. After development, the plates were air dried and sprayed with the revealers in a fume hood.

Total phenolic content estimation

The total phenolic content was determined by Folin-Ciocalteu method [15]. 2 ml of samples (1 mg/mL) or standard were mixed with 1 mL of Folin Ciocalteu reagent (1: 10 v/v) and 1 mL of (7.5g/ml) Na_2CO_3 was added. The mixtures were incubated for 30 min at room temperature and protected from light for subsequent reading of absorbance against a blank solution consisting of methanol. They were read in a 765 nm spectrophotometer and the total phenolic content was calculated using gallic acid as reference in the range of 10- 50µg/ml. The results were expressed in mg of gallic acid equivalents per 100mg of dried extract.

Total flavonoids content estimation

The flavonoid contents were measured by aluminum chloride colorimetric method [16]. 3 mL of samples (1 mg/mL) or standard were added to 1 mL of 2%

methanolic $AlCl_3$. After 15 min incubation at room temperature, the absorbance was measured against a blank of methanol and aluminum chloride in a spectrophotometer at 420 nm. Flavonoid content was estimated using a quercetin standard curve (5-25 µg/ml) and the results were expressed as mg of quercetin equivalent per 100mg of dried extract.

***In-vitro* antibacterial activity**

The well diffusion method was used to determine the antimicrobial activity of the extract prepared from the *Acacia catechu* (L.F.) willd using standard procedure [17].

Results and Discussion

The percentage yield value of hydroalcoholic extract obtained by maceration is presented in table 1. Study revealed that hydroalcoholic solvent commonly used for extraction of antibacterial compounds [18, 19]. The phyto-chemical screenings was performed on hydro alcoholic extracts of *Acacia catechu* (L.F.) Willd. The results are presented in table 2.These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. Preliminary phytochemical screening experiments are commonly performed to promote a guidance of substantial phytochemicals that may be involved in the antibacterial activity of plant extracts. TLC indicated the presence of different types of secondary metabolites, namely gallic acid and quercetin. The results are presented in table 3 and fig 1&2. The content of total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.011X+0.011$, $R^2= 0.998$,** where X is the gallic acid equivalent (GAE) and Y is the absorbance table 4 & fig 3. Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: **$Y=0.040X + 0.009$, $R^2=0.999$**, where X is the quercetin equivalent (QE) and Y is the absorbance table5 & fig 4. Total phenolic content and total flavonoids content was found to be 6.19 and 2.84 mg/100gm respectively table 6. In present investigation the antibacterial activity of extract obtained from the *Acacia catechu* (L.F.) willd were evaluated against pathogens. The fresh pure 100% extracts obtained from plant used to suitably dilute upto the concentrations of 100, 50 and 25 mg per ml and applied on to the test organism using well diffusion method. Results of the experiment are being concluded in the table 7 & 8, which clearly shows the antibacterial activity of extracts. According to the results, the extract of *Acacia catechu* increased the antibacterial effects against the aforementioned microbes. However, the mechanisms of the antibacterial activities of these medicinal herbs remain unclear, while their antibacterial and antifungal effects could be attributed to the presence of phenolic compounds [20-23]. Hence more studies are required to isolate and identify these bioactive compounds responsible for such activities so as to assess their antimicrobial activity *in vivo*.

Conclusion

In conclusion, findings of the present study regarding the features and properties of *Acacia catechu* could be beneficial for researchers and pharmaceutical manufacturers and it is recommended that these compounds be used in toothpastes and antimicrobial drugs in order to prevent various bacterial infections. It is worth mentioned that extraction technique and the choice of solvent could affect the extraction of active principle and hence, affect the results. Continuous and progressing researches need to be conducted to prove the safety, efficiency and to determine the types of compounds responsible for the antimicrobial effects of *Acacia catechu.*

Acknowledgment

The authors are very much thankful to the Scan Research Laboratories, Bhopal-462021, (M.P.) India for carrying out this research work.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper

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Table 1: Percentage yield of extract by maceration (%w/w)

S. No	Percentage yield (%w/w)
1	12.08

Table 2: Phytochemical screening of extract

S. no.	Constituents	Hydro alcoholic extracts
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Wagner's test	-ve
	Mayer's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	General glycosides test	-ve
3.	**Flavonoids**	
	Lead acetate test	+ve
	Shinoda test	+ve
5.	**Tannins and Phenolics**	
	5% $fecl_3$ test	+ve
6.	**Amino acids**	
	Ninhydrin test	-ve
7.	**Cabohydrates**	
	Molichs test	+ve
8.	**Diterpines**	-ve

-ve= Negative, +ve= Positive

Table 3: TLC of extracts

S. No	Extracts	Toluene: Ethyl acetate: Formic acid (5:4:1)	Toluene: Ethyl acetate: Formic acid (7:5:1)
		Quercetin	Gallic acid
1.	Hydro alcoholic	0.96	0.846

Table 4: Calibration curve of gallic acid

S. No.	Concentration	Absorbance
0	0	0
1	10	0.135
2	20	0.247
3	30	0.364
4	40	0.474
5	50	0.581

Table 5: Calibration curve of quercetin

S. No.	Concentration	Absorbance
0	0	0
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815
5	25	1.021

Table 6: Estimation of total phenolics and total flavonoids content

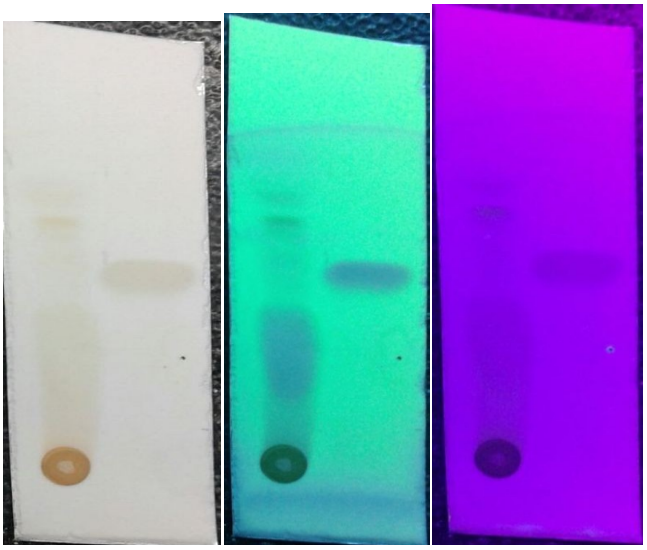
S. No	Extracts	Total phenolic content (mg/100gm of dried extract)	Total flavonoids Equivalent to Quercetin mg/ 100 mg of dried extract
1	***Acacia catechu* (L.F.)**	6.19	2.84

Table 7: Antimicrobial activity of standard drug on microbes

S. No.	Name of drug	Microbes	Zone of inhibition		
			30 µg/ml	20 µg/ml	10 µg/ml
1	Ofloxacin	*S. mutans*	17±0.19	15±0.13	12±0.15
2.	Fluconazole	*Candida albicans*	28±0.11	20±0.09	16±0.04

Table 8: Antimicrobial activity of *Acacia catechu* (L.F.) willd

Extract	Name of microbes	Zone of inhibition		
		100mg/ml	50 mg/ml	25mg/ml
***Acacia catechu* (L.F.) willd.**	*S. mutans*	17±0.5	12±0.57	10±0.5
	Candida albicans	15±0.74	10±0.86	08±0.57

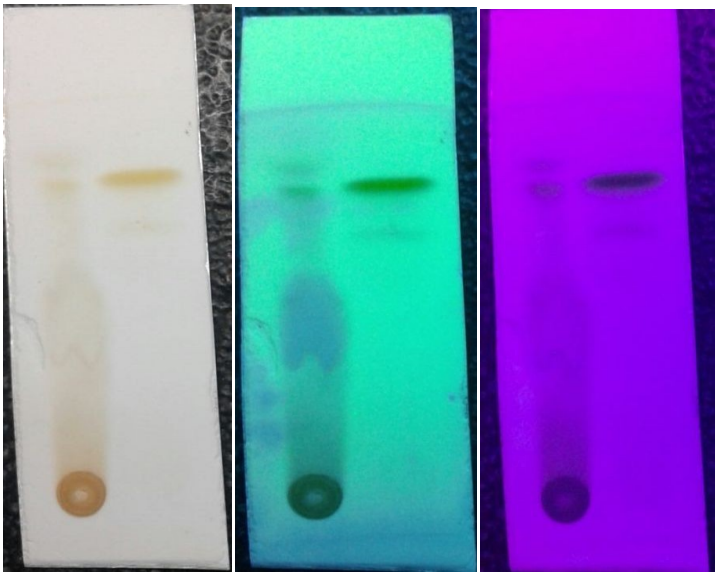


Normal Light Short U.V Long U.V

Spot -1 Hydroalcoholic extract of *Acacia catechu* (L.F.) Wild.

Spot -2 Gallic acid

Figure 1: Photograph of T.L.C. (Gallic acid)



Normal Light **Short U.V** **Long U.V**

Spot -1 Hydroalcoholic extract of *Acacia catechu* (L.F.) Wild.

Spot -2 Quercetin

Figure 2: Photograph of T.L.C. (Quercetin)

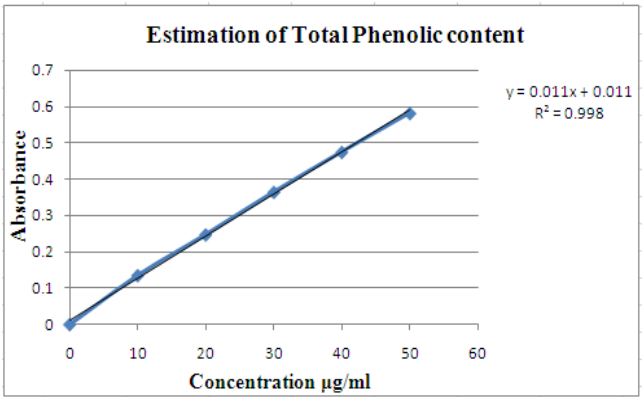


Figure 3: Estimation of total phenolic content

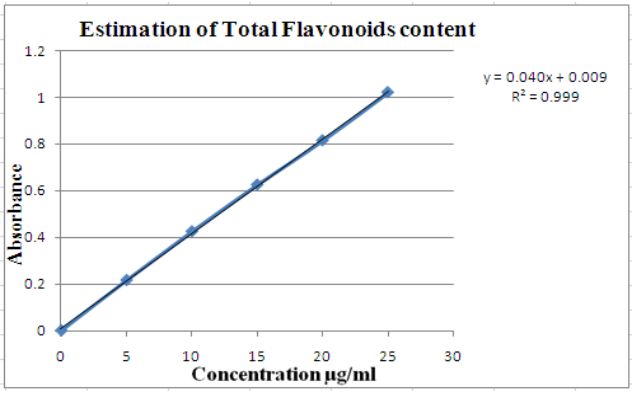


Figure 4: Estimation of total flavonoid content