International Journal for Modern Trends in Science and Technology, 9(05): 74-80, 2023

ISSN: 2455-3778 online
DOI: https://doi.org/10.46501/IJMTST0905011
Available online at: http://www.ijmtst.com/vol9issue05.html

Total Phenolic Content, Anti-Oxidant and Anthelmintic Screening of Selected Medicinal Plants

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To Cite this Article
Karunasree Varicola, Praveen Reddy, Bala Yaswanth Kumar Sunnapu and Jothirmayeedevineni. Total Phenolic Content, Anti-Oxidant and Anthelmintic Screening of Selected Medicinal Plants. International Journal for Modern Trends in Science and Technology 2023, 9(05), pp. 74-80. https://doi.org/10.46501/IJMTST0905011

Article Info
Received: 14April 2023; Accepted: 03 May 2023; Published: 07 May 2023.

ABSTRACT

The purpose of herbal medicine is to bring back the lost homeostasis in the body so that it rejuvenates itself.It uses natural sources to treat various illnesses. Worldwide usage of medicinal plants is tremendously increasing. In the present work, some of the medicinal plants having antioxidant, antimicrobial and anthelmintic potentials were investigated. For this purpose various parts of*Annona squamosa* (custard apple), *Zizypus jujube* (jujube), *Bixa orellana*were selected due to their inherentvaluable therapeutic constituents. Antioxidant activity was estimated by measuring the reducing power and Total phenolic activitywasdetermined byFolin-Ciocalteu's method.The extracts showed significant antioxidant potential, with the alcohol extractof*Annona squamosa* peel exhibiting highest activity and lowest activity was recorded with alcohol extract of *Zizypus jujube*seeds. Of all the tested extracts, Total phenolic content was highest in aqueous extract of *Bixa orellana* peels (13.2±0.30 mg GAE/g) as compared to least activity recorded with aqueous extract of *Bixa orellana* seeds (2.6± 0.25mg GAE/g). Anthelmintic activity was evaluated using Indian earth worms as experimental animals. Aqueous extract of *Annona squamosa* seeds recorded shortest time for paralysis (31.1±0.38 minutes) and death (37.3±0.73minutes) whilethe aqueous extract of *Zizypus jujube* peelsrequired longest time for paralysis(65.9±0.35 minutes) and death (71.6±0.81 minutes). Our experimental results substantiated the traditional uses of selected plant extracts for anthelmintic and anti oxidant activities. It can be concluded that *invivo* studies can afford a strong basis for these findings.

Key words: Anthelmintic, Antioxidant, *Annona squamosa, Zizypus jujube,Bixa orellana* etc.

1. INTRODUCTION

Herbal medicine is sometimes referred to as botanical medicine or herbalism and it involves the use of plants or parts of plants to treat injuries or illnesses. Mammalians have evolved a defense system against free radicals, in which antioxidants perform different roles. In human diseases, 'oxidant–antioxidant' balance is tilted in favor of the reactive species, so that oxidative damage levels increase. Research and clinical trials have helped to shape the field of medicine, and the future for herbal anti-oxidants looks bright. In the present work, few medicinal plants having antioxidant, antimicrobial

and anthelmintic potentials were investigated. For this purpose we have selected leaves, peel, pulp & seeds of *Annona squamosa* (custard apple), *Zizypus jujube* (jujube), *Bixa orellana* fruits which contains valuable therapeutic constituents.

The major chemical constituents of *Annona squamosa* includes alkaloids, others are oxophoebine, reticuline, isocorydinemethylcorydaldine and the flavonoid quercetin-3-o-glucoside and diterpenoid alkaloid atisine the most abundant alkaloid present in the root[1].Custard apples contain anti-oxidants like *Vitamin C*[2].*Ziziphus jujube* are rich in triterpenic acids, nucleosides, flavonoids, phenolic acids, cerebrosides, sugars and amino acids[3].The fruit and its seeds are used in Chinese, Korean and in Kampo[4] medicine. Studies have shown that jujube's high saponin contents has ability to act as a natural sedative and produces a soothing effect on the entire nervous system[5]. *Bixa orellana* oil is rich in constituents of tocotrienols, beta-carotene, essential oils, saturated and unsaturated fatty acids, flavonoids and vitamin C[6].

2. MATERIALS AND METHODS

All chemicals and reagents used were of analytical grade.

Extraction

Selected fruits of *Annona squamosa and Zizypus jujube* were purchased from the nearby fruit market in Vijayawada. The fruits of *Bixa orellana* were collected from forest areas of Srikakulam A.P. The plant materials were authenticated in the department of Botany, Siddhartha college of Arts and Science, Vijayawada, A.P and voucher specimens with codes 2020-AS-0018,2020-ZJ-0019 and 2020-BO-0020 are stored in our college museum. The peels, seeds, pulp, leaves of custard apple ; peel and seeds of jujube ; seeds of Bixa weredried under shade and coarsely powdered using a household grinder and stored in air tight containers.100gm of coarsely powdered drug material was subjected to maceration and crude extracts were collected.After extraction with methanol, the dried marc was extracted with water in a soxhlet apparatus. The crude extracts obtained from maceration and soxhlet were concentrated in a rotary vacuum evaporator and the residue was weighed. %Yield was calculated. They were packed into containers and stored in desiccators until use. The extracts were labeled as mentioned below:

S.NO	NAME OF EXTRACT	Alcohol Extracts	Aqueous Extracts
1.	*Annona squamosa* Pulp	ASPUA	ASPUAq
2.	*Annona squamosa* Peel	ASPA	ASPAq
3.	*Annona squamosa* Seed	ASSA	ASSAq
4.	*Annona squamosa* Leaf	ASLA	ASLAq
5.	*Zyzipus jujube* Peel	ZJPA	ZJPAq
6.	*Zyzipus jujube* Seed	ZJSA	ZJSAq
7.	*Bixa orellana* Peel	BOPA	BOPAq
8.	*Bixa orellana* Seed	BOSA	BOSAq

Preliminary phytochemical screening

Preliminary phytochemical screening was performed following standard procedures[7].

Antioxidant activity:

Total phenolic content:

The concentration of phenolic components in the tested plant extracts was determined using spectrophotometric methodof analysis using Folin-Ciocalteu's reagent[8-10]. Methanolic and aqueous extracts (1 mg/ml) were used in this analysis. The reaction mixture was prepared by mixing 0.5 ml of the extract, 2.5 ml of 10% Folin-Ciocalteu's reagent and 2.5 ml of 7.5% Sodium Bicarbonate. Blank was concomitantly prepared with 0.5 ml of methanol, 2.5 ml of 10% Folin-Ciocalteu'sreagent and 2.5 ml of 7.5% of $NaHCO_3$. The samples were incubated in a thermostat at 45^0C for 45 min. Absorbance was determined at a wavelength of 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for standard solutions of Gallic acid (GA) and the calibration line was construed. Based on the measured absorbance, concentration of phenolics was read (mg/ml) from the calibration line and the content of phenolics in extracts as expressed in terms of gallic acid equivalent (mg of GA/g of extract) was determined. Statistical analysis was performed.

Reducing power:

Reducing power of the extracts was determined by the method of Athukorala.et,al[11-13]. Different concentrations of the extract (20µg/ml - 60µg/ml) were prepared.1ml of the extract was mixed with 2.5ml of Phosphate buffer (200mM, pH 6.6) and 2.5ml of Potassium ferricyanide (30mM) and incubated at 50^0C for 20 min. Thereafter, 2.5ml of Trichloroacetic acid (600mM) was added to the

reaction mixture followed by the addition of 2.5ml of distilled water and 0.5ml of $FeCl_3$ (6mM) and absorbance was measured at 700nm. Ascorbic acid was used as a positive control.

Anthelmintic activity:

Adult Indian earthworms (*Pheretima posthuma*) were used to study anthelmintic activity following standard procedures[14-16]. Earthworms were collected from water logged areas and washed with normal saline to remove soil and fecal matter. Healthy earthworms of 5-8 cm length and 0.2-0.3 cm width were used in the experimental protocols. Samples were prepared by dissolving 2.5 gm of crude extract in 25 ml of 1% gum acacia solution prepared in normal saline. 50 and 100 mg/ml concentrations of each extract were used in the study. Standard drug, Albendazole 50 mg/ml was prepared in 1% gum acacia (prepared in normal saline solution). Along with treatment groups, a control group was also used in the study. The samples were taken in petri plates and adult healthy earth worms (n=3) were introduced. Observations were made for the time taken to induce paralysis and time taken to kill individual worms. Paralysis was said to occur when the worms do not revive even when introduced into normal saline. Death was concluded when worms lost motility followed by fading of their body color.

Evaluation of antimicrobial activity

Preparation of nutrient agar medium:

Required quantities of beef extract, peptone and Sodium chloride were dissolved in 100ml of distilled water and the solution was made upto 200ml with distilled water. The pH of the medium was adjusted to 7.2. Agar was dissolved in above solution and made upto 1000ml with distilled water. 25ml of this solution was transferred into boiling tubes and sterilized in an autoclave at a temperature of 121˚C and a pressure of 15 lbs/sq.inch for 20minutes.

Organisms used:

1. *Bacillus subtilis*
2. *Escherichia coli*

Cup plate method

The media was inoculated at 1% level with 24hrs old culture of the above mentioned test organisms and transferred into sterile petri dishes by standard procedure[17,18]. The medium in the plates was allowed to set at room temperature for about 15-20min. Cups were bored using sterilized borer (0.9mm) in the solidified media. Three cups were bored per each plate. The cups were filled with two dilutions of test i.e. 100µg/ml and 200µg/ml; standard (Streptomycin) 100µg/ml solutions using micro pipette and the plates were incubated for a specific period of 24hours at37˚C. All the tests were run in triplicate. The zones of inhibition were measured in millimeters and the anti-microbial activity was compared with the standard drug.

Statistical analysis: All the experiments were completely randomized. Statistical significance was determined among various treatments using Graph pad prism 9.5.1 software. A statistical significance of $p<0.05$ was considered to be significant.

3. RESULTS AND DISCUSSION

Percentage yield:

$$\text{Percentage yield}=\frac{Weight\ of\ extract\ *100}{Weight\ of\ crude\ drug}$$

Highest yield among aqueous and alcohol extracts is obtained from ZJPAq (16.41%W/W)and ASPEA(36.2%W/W) indicating polar components are rich in these extracts. % yield details of various extracts are given in table 1.

Preliminary phytochemical screening:

From the preliminary phytochemical screening, it is evident that peel, seeds, leaves, pulp extracts of *Annona squamosa* and the peel, seed extracts of *Zizypus jujube* and *Bixa orellana* were found to contain important phyto constituents like carbohydrates, alkaloids, glycosides, tannins, gums and mucilage. These constituents are reported to possess several therapeutic uses[19].There is an undoubted correlation between phytoconstituents of a plant and their therapeutic applications. The resultsof preliminary screening are given in table 2.

Reducing power:

The present study was undertaken to evaluate anti-oxidant activity of different aqueous and alcohol extracts of *Annona squamosa, Zizypus jujube* and *Bixa orellana.* Among the alcohol extracts, highest and lowest reducing power was expressed inASPA and ZJSA respectively as compared to the Standard. In the aqueous extracts, highest reducing power was observed with ASPAqas compared to the standard at a concentration of 60mcg/ml. ASPUAq, BOSAq, BOPAq, and

ZJPAqextracts also exhibited remarkable reducing power. ASLAq extract exhibited lowest activity. Among the aqueous and alcohol extracts,highest and lowest reducing power is recorded with ASPA and ZJSA. Injuries caused by free radicals are an important factor in many pathological and toxicological processes[20]. Oxidative stress is characterized by the inability of endogenous antioxidants to counteract oxidative damage to biomolecules and also plays a key role in the pathophysiology of a variety of diseases[21-23]. Reducing power is associated with the antioxidant activity and may serve as significant reflection of its protective role. Compounds with reducing power indicate that they are electron donors and can reduce the lipid peroxidation process, so they act as primary and secondary antioxidants. Our findings are supported by previous works reporting antioxidant property in the selected plants[24-26].The results of this study are shown in figures 1 & 2.The hierarchy of reducing power of corresponding extracts are as follows:

Alcohol extracts:ASPA>Std>ASLA>ZJPA>ASSA>BOPA>ASPUA >BOSA>ZJSA

Aqueous extracts:ASPAq>ASPUAq>BOSAq>Std>BOPAq & ZJPAq>ZJSAq>ASSAq>ASLAq

Anti-microbial activity

In the tested range, the extracts of *Annona squamosa*, *Zizypus jujube* and *Bixa orellana* did not exhibit any positive results (zone of inhibition).

Total phenolic content:

Total phenolic content of the tested extracts was in the range of 2.6± 0.25(BOSAq) to 13.2±0.30(BOPAq) mg GAE/g. Plant polyphenols, a diverse group of phenolic contents (Flavonols, Anthocyanins, Phenolic acids etc.) possess an ideal structural chemistry for free radical scavenging activity[27]. Anti-oxidative properties of the polyphenols arise from their high reactivity as hydrogen or electron donors, ability to stabilize and delocalize unpaired electrons and their potential to chelate the metal ions. Phenolic compounds are reported to possess antioxidant, anticancer, antibacterial, cardioprotective, anti-inflammatory, skin protecting and immune system protecting properties[28,29]. The total phenolic content of *Annona squamosa, Bixa orellana* and *Zizypus jujube* extracts is depicted in figure 3.Our experimental results on Total Phenolic content correlated with those obtained by other researchers working on these selected plants [30,31].

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Anthelmintic activity:

All the extracts were tested for their ability to paralyze and/ or to kill the Indian earth worms during the study period. ASSAq extract was found to possess anthelmintic activity comparable to the standard drug Albendazole. This extract paralyzed the test organisms in 31.1±0.38 minutes and death resulted in37.3±0.73minutes, whereas, the standard drug paralyzed the test organisms in 30.5±0.29 minutes and death occurred in 39.9±0.20minutes. ASPA (paralysis time 31.4±0.58 and death time 40.6±0.54) and ZJSAq (paralysis time33.4±0.26 and death time 41.6±0.55) also possessed demonstrable anthelmintic activity.It is also observed that among all tested extracts, ASSAq produced paralysis and death in shortest time while ZJPAq has taken longest time for paralysis(65.9±0.35) and death(71.6±0.81). There existed an inverse relationship between potency of the extracts and time taken for paralysis /death of the worms. The control group animals were alive up to 24 hrs.The results are shown in figure 4. Different mechanisms by which chemotherapeutic agents act as anthelmintics are by disruption of neuromuscular physiology, blockade of energy metabolism and by disrupting reproductive system[32].Substantial evidence for anthelmintic activity in the selected plants is also reported by few other researchers[33-35].

Table 1: Percentage yield of extracts

S.NO	NAME OF EXTRACT	YIELD			
		Alcohol Extract	**%YIELD**	**Aqueous extract**	**%YIELD**
1.	*Annona squamosa* Pulp	ASPUA	21.2	ASPUAq	9.41
2.	*Annona squamosa* Peel	ASPA	36.2	ASPAq	3.494

3.	*Annona squamosa* Seed	ASSA	5.66	ASSAq	1.466
4.	*Annona squamosa* Leaf	ASLA	22.1	ASLAq	7.85
5.	*Zyzipus jujube* Peel	ZJPA	18.5	ZJPAq	16.41
6.	*Zyzipus jujube* Seed	ZJSA	4.1	ZJSAq	0.901
7.	*Bixa orellana* Peel	BOPA	6.53	BOPAq	4.41
8.	*Bixa orellana* Seed	BOSA	2.3	BOSAq	4.62

Table 2: Preliminary phytochemical screening of extracts

S.NO	Extract	Alkaloids	Carbohydrates &Glycosides	Gums &mucilage	Proteins & amino acids	Tannins &Phenolic compounds	Flavonoids
1	ASPUA	-	+	+	-	+	-
2	ASPUAq	-	+	+	-	+	-
3	ASPA	+	-	+	-	-	-
4	ASPAq	+	+	+	+	+	-
5	ASSA	+	+	-	-	-	-
6	ASSAq	+	+	+	-	-	-
7	ASLA	-	+	+	-	+	-
8	ASLAq	-	+	+	-	+	-
9	ZJPA	+	+	+	-	+	-
10	ZJPAq	+	+	-	+	-	-
11	ZJSA	-	+	-	-	-	-
12	ZJSAq	+	+	+	-	-	-
13	BOPA	-	+	-	-	+	-
14	BOPAq	+	+	-	-	-	-
15	BOSA	+	-	+	-	-	+
16	BOSAq	-	+	+	-	+	+

+ = (POSITIVE) - = (NEGATIVE)

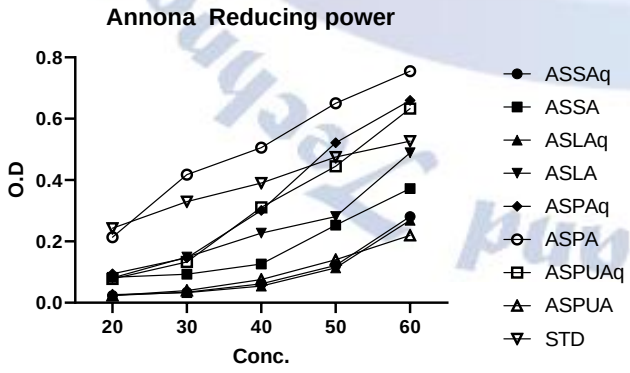


Figure 1: Reducing power of*Annona squamosa* extracts

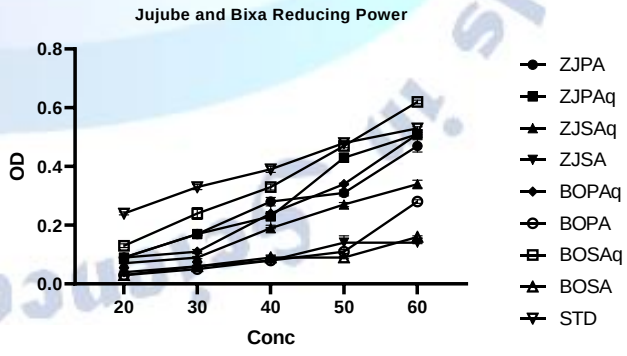
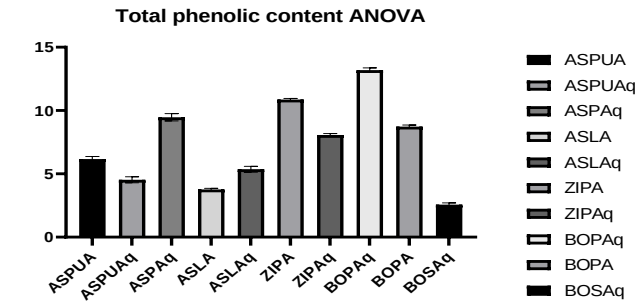


Figure 2: Reducing power of*Zizypus jujube and Bixa orellana* extracts



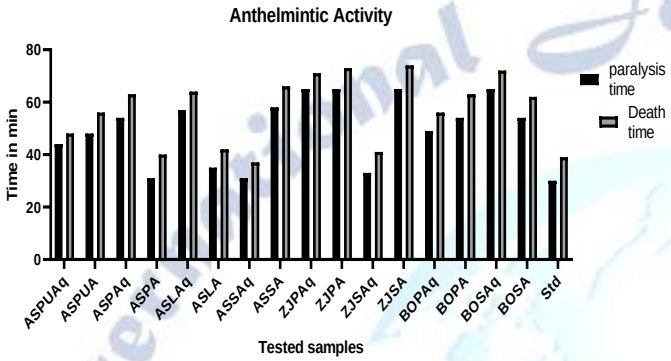


Figure 3: Total phenolic content Figure 4: Anthelmintic activity.

4. CONCLUSION

Medicinal plants have been the base for treatments through much of human history and such traditional medicines are still widely practiced today in the present society. Our experiments proved that alcohol and aqueous extracts of fruit peel, pulp, seeds, and leaves of*Annona squamosa*, *Zizypus jujube* and *Bixa orellana* contain carbohydrates, alkaloids, glycosides, tannins, flavonoids as chief principles. The potential antioxidant activity and anthelmintic activity of these extracts was comparable to the standard drugs used. Further, these extracts showed no antibacterial activity in the tested range. It can be concluded that *in vivo* studies need to be taken up to give a strong basis for the *invitro* findings.

Acknowledgments

The authors wish to thank the management of K.V.S.R. Siddhartha College of pharmaceutical sciences, Vijayawada, for providing the facilities for successful completion of the work.

Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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