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Preliminary Phytochemical and Anti-Bacterial Studies on *Passiflora edulis*

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Abstract

Preliminary phytochemical and antibacterial activity of the leaf and callus of *Passiflora edulis* Sims, were examined using extracts of benzene, methanol, ethanol, isopropanol, chloroform and petroleum ether. Of these, chloroform extract of the leaf and callus showed the maximum solubility and antimicrobial activity with the MIC ranging from 100 to 250 µl. Extracts of benzene, petroleum ether and isopropanol were ineffective in inhibiting the selected bacteria. In addition, this phytochemical study confirmed the presence of alkaloids, saponins, tannins and triterpenes from ethanol and chloroform extracted sources.

Key Words: *In vitro, in vivo*, calli, bio-efficacy, antibacterial, phytochemistry.

Introduction

Each and every human civilization on earth has been rooted in the biodiversity of nature. Biodiversity provides humankind enormous direct benefits and indirect essential services through natural ecosystem function and stability. It comprises about 5 to more than 50 millions of species of which 270,000 are plant species. The World Health Organization (WHO) estimates that up to 80% of the world's people rely on plants for their primary health care. Plants contain chemical constituents such as tannins, flavonoids, steroids, saponins, glycosides, phenolics, terpenes, alkaloids, waxes, essential oils, carbohydrates, amino acids, proteins etc[1]. The presence or absence of some of these constituents has been found useful in the placement of the plants in their taxonomic categories.[2] Particular chemical constituents or drugs are found to be present in particular plants or plant parts that are used for many useful purposes.[3 - 7] A special feature of angiospermic plants is their capacity to produce a large number of organic chemicals of high structural diversity. These so-called secondary metabolites have contributed more than 7000 different compounds in use today as cardiac drugs, anticancer agents, hormones, antibiotics, laxatives, diuretics, analgesics, anesthetics, drugs for ulcer treatments and antiparasitic compounds. In the USA, 74% of drugs are based on plants.[8] A large portion of the world population, especially in developing countries like India depend directly on the traditional medicine system for a variety of diseases. Approximately 20% of the plants found in the world have been submitted for pharmacological and biological screening.[9] Several thousands of plants (approximately 20000 by traditional practice) are used medicinally, mainly as herbal preparations in the indigenous system of medicine in India and the sources of very potent and powerful drugs which have stood the test of time and modern chemistry has not been able to replace most of them. The systematic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with potential to act against multi-resistant

pathogenic bacteria and fungi.[10] The accumulation of phytochemicals in the plant cell cultures has been studied for more than thirty years, and the generated knowledge has helped in the realization of using cell cultures for production of desired phytochemicals.[11] The horticulturally important angiospermic plant *Passiflora edulis*, a climber belonging to the family Passifloraceae, is distributed in many tropical countries. The plant is cultivated for ornamental purposes and for its fruit value. A lot of reports are available on phytochemical, anti–microbial, anti-fungal, anti–viral and anti–bacterial activities, but there is no report for *Passiflora edulis*. Based on this background the present study was intended to screen the plant *Passiflora edulis* for the presence or absence of phytochemicals and antibacterial activities. In addition, another aim was to compare the potential values of *Passiflora edulis* leaves and *in vitro* derived calli.

Materials and Methods

The plant *Passiflora edulis* Sims was collected from Main campus botanical garden of Bahir Dar University, Ethiopia and identified with the help of standard flora. For callus induction, the young shoots were washed with running tap water for 10 minutes and surface sterilized with 0.1 (w/v) $HgCl_2$ solutions for one minute and rinsed thrice with distilled water. The leaves stem nodes and internodes were cut into smaller segments (1cm) and used as explants. The explants were placed horizontally on solid basal Murashige and Skoog[12] (MS) medium supplemented with 3% sucrose, 0.6% (w/v) agar (HIMEDIA, Mumbai) and different concentrations and combinations of 2, 4-D, BAP, Kin, NAA and IAA aseptically. The pH of the medium was adjusted to 5.8 before autoclaving at 121^{o}C for 15 min. The cultures were incubated at 25$\pm$2^{o} C under cool fluorescent light (2000 lux 14 hr photoperiod). The callus cultures were maintained for a period of over 10 months by periodic sub-culturing with 2 to 4 weeks intervals on to fresh medium. Phytochemical assays were performed on the 21st day. Consequently, the calli were harvested at the transfer age of 3 to 4 weeks and kept at above 90°C for 3 to 5 minutes at hot air oven to inactivate the enzyme activity followed by continuous drying at 50 to 60°C for 60 to 72 h. Both dried leaf and calli were homogenized to a fine powder and further exploited for extraction, phytochemical and bacterial efficacy analysis. Dried leaves and *in vitro* derived callus were powdered using the electric homogenizer and exhaustively extracted with 150 ml of solvent (benzene, methanol, ethanol, isopropanol, chloroform and petroleum ether) for 8 - 12 h by using the soxhlet apparatus. The preliminary phytochemical screening was performed according to the Harborne [13 & 14] method and the results are tabulated in Table 1. The extracts were concentrated and these extracts were subjected for their bio-efficacy against the selected bacteria. Stock cultures were made fresh every seven days on agar slants during this scheme of work. Pure bacterial cultures, namely *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella aerogenes, Aeromonas sps*, *Serratia* and *E. coli* were maintained on nutrient broth at 37°C for 24 hrs. Different concentrations of extracts ranging from 100 to 250 µl were used for bacterial sensitivity test. Antibacterial efficacy was performed by well - diffusion method and incubated for 24 hrs at 37° C[15]. The inhibition zone and bio-efficacy activity against the bacteria were recorded. The experiments were repeated in triplicate and the results are presented in Table 2.

Results and Discussion

The results of phytochemical and anti–bacterial screening tests on extracts of leaf and calli of *Passiflora edulis* in different solvents against pathogenic bacteria using diffusion techniques are depicted in Tables 1 and 2. The solvent chloroform showed maximum extraction value and its extract showed the maximum bio-efficacy when compared

with other solvents due to the presence of variety of compounds such as saponins, steroids, tannins, phenolics, triterpenoids, alkaloids and flavonoids (Table 1). Alkaloids are widely distributed and naturally occurring in plants. Most of the alkaloids from plants are used for medicinal purpose. Alkaloids are chemical constituents from plants that can work on the nervous system of the human body and used for analgesic, antispasmodic and bacterial effects[16 & 17]. In the present study also we confirmed the presence of alkaloids in all the plant extracts and that augments the use of alkaloid in global pharmaceutical market. Tannins are antiseptic in nature; they have astringent properties and can hasten healing of wounds in a flamed membrane.[18] They are free from the attack of parasitic fungi and insects. Tannins can be used in this capacity to give the human body a resistance against parasites.[19] Tannic acid has been found to have anti-bacterial, antiseptic, astringent, antiulcer and antiviral properties.[20] Flavonoids, on the other hand, are potent water–soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity.[21 – 24] Flavonoids in intestinal track lower the risk of heart disease. As antioxidants flavonoids from these plants provide anti-inflammatory activity.[23] Saponin possesses specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include antimicrobial,[25] anti–inflammatory,[26] anti–feedent,[27] and hemolytic effects.[28] The results of the present study revealed that, antibacterial efficacies of chloroform, ethanol, methanol, isopropanol and petroleum ether extracts were varied in effectiveness which may be attributed to the presence of tannin and saponins. The presence of phenolic compounds in the plant indicates the anti–microbial potential. In the present study, we also observed the anti bacterial activity, which agrees with the findings of Ofokansi et al.. [29 & 24] Both benzene and petroleum ether extracts were found to be ineffective of bacterial inhibition, due to the presence of less numbers of active compounds, saponins, steroids and alkaloids. Our observations were supported by previous observations on *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts.[10] The chloroform extracts of leaf and callus inhibited growth of all the tested bacteria (Table 2). Petroleum ether, isopropanol and benzene extracts showed the minimum zone of inhibition against the selected bacteria. The benzene and isopropanol extracts exhibited no activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In addition, our benzene extract showed almost no activity against *Aeromonas* and *Serattia*. The bio-efficacy analysis confirms the phytochemical observations. The other aqueous fractions were active against all the test bacteria, but the efficacy was varied (Table 2). The chloroform extract showed the maximum efficacy against the tested bacteria. The ethanolic extract showed maximum efficacy against *E. coli* compared to other fractions. Methanolic and chloroform extracts showed the maximum inhibition zone against *Staphylococcus aureus* and *Serratia*. The extracts of higher plants have been proved a very good source of antibiotics against various bacterial agents.[30] Plant-based antimicrobial compounds have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Results of the present study are found directly correlated with the observations of previous workers.[3,4,10,11& 15] The observations obtained in the present study also added a note on the phytochemical history. Earlier observations on *Baliospermum axillare, Mimosa hamata* and *Nerium oleadaner* leaf and callus extracts showed considerable anti-bacterial and anti-microbial activity.[9, 10&31] The present observation augments the previous phytochemical and bio-efficacy studies on cell cultures. It is noteworthy that varied fractions obtained from the callus also exhibited similar pattern of bioactivity against all tested bacteria. The efficacy was, however, lower.

The present observations coincided with Jain et al.[3] observation on *Mimosa hamata*. The observations of the present study strengthen the bio-efficacy studies on cell cultures. Further work is required to find out the active principle from the plant extracts and to carry out pharmaceutical studies.

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Table 1. Preliminary Phytochemical Screening of *Passiflora edulis*.

S. No	Experiment	*In vivo* leaves and *in vitro* leaves derived Calli - Extraction from different solvents					
		Benzene	**Ethanol**	**Chloroform**	**Isopropanol**	**Methanol**	**Petroleum Ether**
1	**Saponin**	Absent	Present	Present	Present	Absent	Present
2	**Steroid**	Present	Present	Present	Present	Present	Present
3	**Catachin**	Absent	Absent	Absent	Absent	Absent	Absent
4	**Amino acid**	Present	Present	Present	Absent	Absent	Present
5	**Tannin**	Absent	Present	Present	Present	Present	Present
6	**Anthro quinone**	Absent	Absent	Absent	Absent	Absent	Absent
7	**Phenolics**	Absent	Present	Present	Absent	Present	Absent
8	**Sugar**	Absent	Present	Absent	Absent	Absent	Absent
9	**Triterpene**	Absent	Present	Present	Absent	Absent	Absent
10	**Alkaloids**	Present	Present	Present	Present	Present	Present
11	**Flavones**	Absent	Absent	Present	Absent	Present	Absent

Table 2. Antibacterial efficacy of *Passiflora edulis*.

Nature of Extract	Conc. in µl	Leaves extract activity against the bacterial inhibition zone in mm						*In vitro* Leaves Derived Callus tissue – extract activity against the bacterial inhibition zone in mm					
		EC	***SA***	***PA***	***KA***	***A***	***S***	***EC***	***SA***	***PA***	***KA***	***A***	***S***
Ethanol	**100**	3	2	±	±	4	3	3	2	±	±	3	2
	125	4	3	±	±	4	4	4	3	±	±	3	4
	150	6	3	1	1	5	6	5	3	±	±	4	5
	175	7	4	1	1	7	7	6	4	±	±	5	5
	200	8	7	1	1	7	7	6	6	±	±	5	5
	225	9	7	1	1	7	7	8	6	±	±	5	6
	250	10	8	1	1	7	7	8	7	±	±	6	6
Methanol	**100**	3	4	1	1	2	2	2	3	±	±	2	2
	125	3	5	1	1	3	4	2	4	±	±	3	3
	150	4	6	2	2	3	5	4	5	2	2	3	4
	175	6	8	2	2	4	5	4	7	2	2	4	5
	200	6	8	3	3	4	8	4	7	2	3	4	6
	225	6	8	4	4	4	8	6	7	3	4	4	6
	250	8	9	4	4	5	9	6	8	3	4	5	7
Chloroform	**100**	4	5	2	3	3	3	3	4	2	3	3	3
	125	4	5	2	3	3	3	4	5	2	3	3	3
	150	4	7	3	3	4	5	4	7	3	3	4	5
	175	6	9	4	3	4	7	5	7	3	3	4	7
	200	7	10	4	4	5	7	5	8	3	4	5	7
	225	7	10	4	4	5	7	6	8	3	4	5	7
	250	8	10	4	4	7	9	6	9	3	4	6	9
	100	4	3	2	3	3	3	3	3	±	2	3	3

Petroleum ether	**125**	4	3	2	3	3	3	3	3	±	2	3	3
	150	4	4	3	3	4	5	3	4	±	3	4	5
	175	6	5	4	3	4	7	6	4	2	3	4	7
	200	7	5	4	4	5	7	7	5	2	4	5	7
	225	7	5	4	4	5	7	7	5	3	4	5	7
	250	8	6	4	4	7	9	8	6	3	4	5	7
Isopropanol	**100**	3	2	±	±	4	3	3	±	±	±	4	3
	125	4	3	±	±	4	4	3	2	±	±	4	4
	150	6	3	±	±	5	6	5	3	±	±	5	6
	175	7	4	±	±	7	7	5	3	±	±	5	5
	200	9	7	±	±	7	7	7	5	±	±	6	6
	225	9	7	±	±	7	7	8	6	±	±	6	6
	250	10	8	±	±	7	7	8	6	±	±	6	7
Benzene	**100**	2	3	±	±	±	±	2	3	±	±	±	±
	125	2	3	±	±	±	±	2	3	±	±	±	±
	150	3	3	±	±	±	±	3	3	±	±	±	±
	175	4	4	±	±	±	±	4	4	±	±	±	±
	200	4	4	±	±	±	±	4	4	±	±	±	±
	225	5	4	±	±	±	±	5	4	±	±	±	±
	250	5	5	±	±	±	±	5	5	±	±	±	±

SA - *Staphylococcus aureus,* PA -*Pseudomonas aeruginosa,* KA - *Klebsiella aerogenes,* A - *Aeromonas sps*, S - *Serratia* EC – *Escherichia coli*