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Evaluation of phytochemical investigation and immunomodulatory activity of four different plant species of vidari by carbon clearance test on wister rats

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Abstract

The present study was undertaken to evaluate immunomodulatory activity of vidari. Vidari is the name of four botanical sources of plant drug which is controversial in Indian market. They are namely; *Pueraria tuberose* DC (P.t) (Family: Leguminosae), *Ipomoea mauritiana* Jacq (I.m) (Family: Convolvulaceae), *Adenia hondala* de Wide (A.h) (Family: Passifloraceae) and *Cycas circinalis* Linn (C.c) (Family: Cycadaceae). In this study, preliminary investigations on phytoconstituents were evaluated separately and revealed the presence of carbohydrate, phenols, alkaloids, glycosides, saponins, phytosterol, flavonoids, wax and gums in all the examined drugs but tannins were absent in all the drugs. Furthermore, immune response was evaluated by carbon clearance assay (Granulopectic index) against standard marketed drug, *Withania somnifera* (W.s), an approved potent immunomodulatory agent (positive control). Wister rats of either sex were divided into 6 groups (6 animal each) and all the drugs were administered at dose of 250 mg/kg body wt. after calculated individual maximum tolerance dose as per OECD guidelines. All the test substances and standard showed significant activity compared to control ($p < 0.001$) and, thereafter, immune response with respect to granulopectic index of P.t ($p > 0.05$) was found better (0.051/ min), compared to other extracts but the value was lesser than that of positive control (0.053/ min). This study ascertained pharmacologically, the authenticity of four different species of plant belong to the same name of "vidari".

Key words: Carbon clearance assay, immunomodulatory, phytochemicals, vidari, *Withania somnifera*

1. Introduction

A vast number of research investigations have described the use of plants in traditional ayurvedic medicine for many years and have recently gained tremendous focus in the field of pharmacological industries. The quality assessments of the biological properties of extracts from various plants can serve as a source of newer drug molecules which help in many areas of health related problems (Acuna *et al*., 2009) and several marketed plant products have been used for the treatment of different diseases (Figueroa *et al*., 2015). Many herbs enlisted as "Rasayana" drugs in Ayurveda are believed to improve defense mechanisms of the body, promote physical and mental health and enhance longevity (Shukla *et al*., 2009; Jantan *et al*., 2011; Kumar *et al*., 2012; Zhuang *et al*., 2012), among them *Ocimum sanctum*, W.s is well established as potent immune response plant drug (Caroline Jeba *et al*., 2011). In order to meet the rising demand for the raw drugs, adulteration and substitution have become frequent which in turn results in compromised quality of herbal medicines. Dried plant products sold in the market are generally difficult to identify and at the same time, identification of first step in quality control of herbal medicines. Different herbal raw drugs are sold under the same name and create controversies with respect to the botanical identities of drugs, available in the market. Among the plants known for medicinal value, the ayurvedic plants with the name of vidari are very important for their therapeutic potentials. Vidari has four different plant species with their respective botanical identities and family (Venkatasubramanian *et al*., 2009), namely; *Pueraria tuberose* DC (P.t) (Family: Fabaceae), *Ipomoea mauritiana* Jacq (I.m) (Family: Convolvulaceae), *Adenia hondala* de Wide (Family: Passifloraceae) and *Cycas circinalis* Linn (C.c) (Family: Cycadaceae). The substitutes may or may not resemble vidari in terms of morphology, properties or actions. Vidari is an ingredient of Chyavanaprash, one of the top-selling products of ayurvedic industry (Venkatasubramanian et al., 2009). As per the Ayurvedic Pharmacopoeia of India correlates vidari to P.t which is commonly known as "Vidarikand". The plant is available throughout the India and it is traditionally used for bleeding disorders, decreased seminal quantity, purification of blood, tuberculosis, cough, pain, burning micturition, herpes, rejuvenator, tonic, restorative, aphrodisiac, galactagogue, diuretic, demulcent, haemorrhage, bronchial asthma and urinary disorders (Yoganarasimhan, 1996; The Ayurvedic Pharmacopoeia of India, 2006; Maji *et al*., 2014). But as per the local names of vidari, it

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contains another three plants and they are used for different purposes like, tubers of I.m, A.h are used against sexual debility, pain, inflammations, burning sensations and skin problems (Puri, 2003) and C.c is used for high blood pressure, headaches, congestion, rheumatism, bone pain and treatment of estrogen-dependent tumors (Maria *et al*., 1995; Kalpashree and Raveesha, 2013). These activities are mainly due to the presence of several phytochemicals present in these plants, *viz*. puerarin, daidzein, genistein, genistein, puerarone, coumarin, anthocyanin, lupinoside, tuberosin, pterocarpintuberosin, puetuberosanol and hydroxytuberosone (Maji *et al*., 2012; Sawale *et al*., 2013) among the phytochemicals, the isoflavones puerarin, daidzein and genistein are most important for their immunomodulatory activity (Sawale *et al*., 2013) but no such concrete evidences are available for determination of the pharmacological activity, especially comparative immunomodulatory activity for all four drugs. Based on the above context, the present study was designed to explore the immunomodulatory potential of all four different plant drugs and to establish the potent pharmacological authentication under the same local name of vidari.

2. Materials and Methods

2.1 Plant material

Dried mature tubers of P.t, I.m, A.h and stems of C.c used for the study were collected by qualified field botanists from different locations of Belgaum, Bengaluru local market (Karnataka), Wayanad, Kozhikode, Idukki (Kerala), Pune, Bheamashanka (Maharashtra) and Tirupati (Andhra Pradesh). The samples were authenticated by qualified plant taxonomists of Herbarium division of FRLHT. Voucher Specimen No. (P.t = L/07/02/032; I.m = L/07/02/035; A.h = L/07/10/027 and C.c = L/07/04/011) for all the samples were preserved at Raw Drug Collection Centre, FRLHT, Bangalore.

2.2 Preparation of extract

Dried coarsely powdered tubers of P.t, I.m, A.h and stem of C.c (250 g) were separately defatted with petroleum ether at 45^{o}C for 5 h, using Soxhlet apparatus. The marc left was subsequently extracted with ethyl acetate, methanol and water (45-50^{o}C) for 7 h. The crude brown residue mass of extract was then concentrated by using rotary flash evaporator and then stored at 4^{o}C in refrigeration condition in separate glass bottles. The percentage yield of extracts were calculated on dry wet basis.

2.3 Pharmacognostic studies

All the above extracted samples were screened for various phytochemical constituents' presence as per the method described by Kokate (1997).

2.4 Experimental animals

Wister rats of either sex were used in the present study. The animals were fed with standard pellet diet, water *ad libitum* and maintained under standard environment condition employed. They were housed under standard conditions (22 ± 5^{o}C with 12 h of light/dark cycle). All experimental protocols were approved by Institutional Animal Ethical Committee Clearance (AACP/IAEC/M-75/2006), Al-Ameen College of Pharmacy, Bangalore (Karnataka), India.

2.5 Maximum tolerance dose (MTD)

All the dried coarse powdered drugs were tested for MTD to determine the nature and extent of the untoward reactions which might follow the administration of a single dose (or overdose) of the drug, using swiss albino mice of either sex (25-30 g).

Mice (fasted for 3-4 h.) were divided into four groups, of two animals (male and female) each for administration of each crude drug. Mice were administered with test substances orally at the dose of 1g/kg body weight. Then the animals were observed for 24 h. for sign and symptoms of acute toxicity, *viz*. mortality, tremors, alertness, eye movements and motility. Further, second dose of 2 g/kg body weight was given to the animals and observed for 72 h., which was also found to be safe and no toxic symptoms were observed. W.s was used as standard at the dose of 250 mg/kg (Davis and Kuttan, 2000) and as per that recommended doses were selected for all tested drugs at the dose of 250 mg/kg.

2.6 Experimental protocol

All the tested animals', randomly divided into 6 groups of 6 rats each.

Group 1: Control animals

Group 2: *Pueraria tuberosa* DC. (250 mg/kg)

Group 3: *Ipomoea mauritiana* Jacq. (250 mg/kg)

Group 4: *Adenia hondala* de Wide (250 mg/kg)

Group 5: *Cycas circinalis* L. (250 mg/kg)

Group 6:*Withania somnifera* (L.) Dunal standard treated animals (250 mg/kg)

2.7 Carbon clearance assay

All the **a**nimals in the 2nd, 3rd, 4th, 5th and 6th group received 250 mg/kg suspension of drugs, P.t, I.m, A.h, C.c and W.s for a period of 5 consecutive days. After 48 hours of last dose of drug, blood sample was collected by retro-orbital puncture which was taken as blank (Tiwari *et al*., 2004; Zhao *et al*., 2005)**.** Each rat received an intravenous injection of carbon ink suspension consisting of 3 ml of pelikan ink (Germany), 4 ml of saline and 4 ml of 3% gelatin solution (Morris *et al*., 2003) *via* the tail vein at a dose of 0.5 ml/100 gm body weight (Daswani and Yegnanarayan, 2002). Blood was collected immediately after i.v injection of carbon ink suspension and at an interval of 3, 6, 9, 12, and 15 min. 25 µl of blood was added to 2 ml of 0.1% sodium carbonate solution to lyse the erythrocytes. At the end of the blood collection, the absorbance of each sample was measured at 660 nm, using spectrophotometer. The clearance value 'K' was calculated according to the following equation:

$$K= (\log OD_1) – (\log OD_2) / t_2 – t_1$$

where 't_1' represents the time in minutes when the samples 'OD_1' were withdrawn.

The mean of the clearance values at different time intervals gives the clearance value K (Granulopectic index) (min^{-1}).

2.8 Statistical analysis

Data were expressed as the mean ± standard deviation (S.D) and statistical analysis was performed using ANOVA, followed by Bornferronis multiple comparison test.

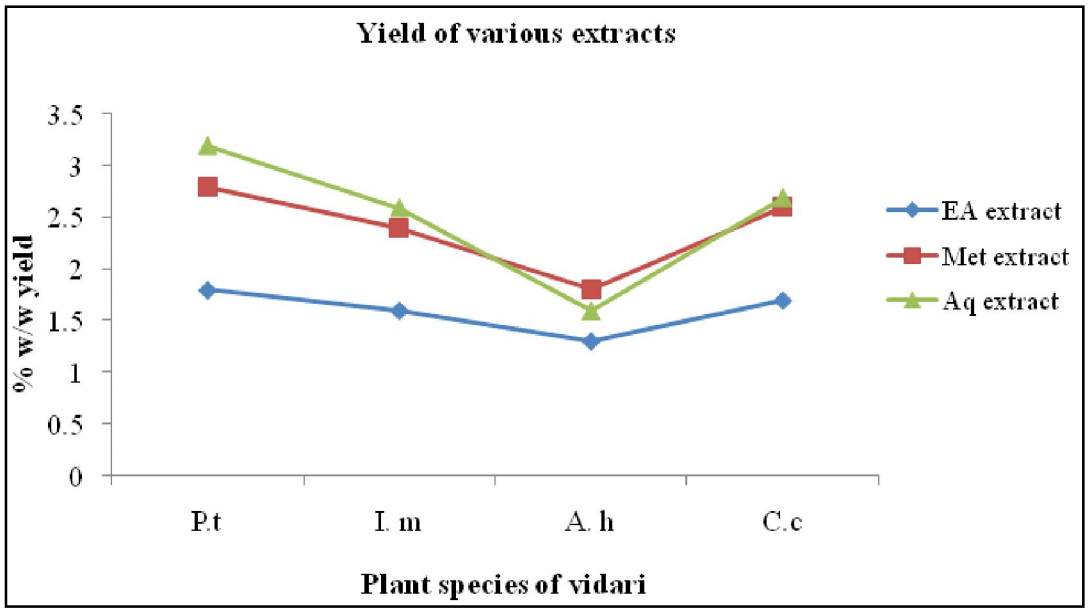
3. Results and Discussion

3.1 Yield of the extracts of vidari plants

Percentage yield of all the extracted drugs were calculated and tabulated in the Figure 1. The results revealed that aqueous extract of P.t yielded 3.2% w/w, followed by methanol extract (2.8% w/w)

whereas aqueous extract of I.m showed 2.6% w/w, followed by 2.7% w/w for C.c. but the yield was less in A.h extracts than all others. A.h aqueous extract showed 1.6% w/w, followed by 1.8 % w/w yield in methanol extract. This result variation may be due to the cultural condition of the plant sources whereas several research references revealed that the yield of the plant drugs are varied with the several conditions, *viz*. source, location, soil nature, parts use harvesting time, session and so on (Evans, 1996; Cavaliere, 2009; Namdeo *et al*., 2010).

Figure 1: Yiled of various extracts for all the different plant species of Vidari



P.t = Pueraria tuberose DC; I.m = Ipomoea mauritiana Jacq; A.h = Adenia hondala de Wide; C.c = Cycas circinalis Linn.; EA = Ethyl acetate; Met = Methanol; Aq = Aqueous

Table 1: Phytochemical study of various plants of vidari

	P. t			I. m			A. h			C. c		
C.C	EA	Met	Aq	EA	Met	Aq	EA	Met	Aq	EA	Met	Aq
Alk	–	+	–	–	–	+	–	–	–	–	–	–
Car	–	+	+	–	+	+	+	+	+	–	+	+
Gly	–	+	+	–	+	+	–	+	+	–	+	–
Sap	–	+	+	–	–	+	–	–	+	–	–	+
Ste	–	+	+	–	+	–	–	+	–	+	+	–
Fats	–	–	–	–	–	–	–	–	–	–	–	–
Res	–	–	–	–	–	–	–	–	–	–	–	–
Fla	–	+	+	–	+	+	–	+	–	–	+	+
Phe	–	+	+	–	+	–	–	–	–	–	–	–
Tan	–	–	–	–	–	–	–	–	–	–	–	–
Pro	–	–	+	–	–	+	–	–	+	–	+	+
Gum	–	+	+	–	–	+	–	–	+	–	+	+

EA = Ethyl acetate; Met = Methanol; Aq = Aqueous
Alk= Alkaloids; Car = Carbohydrates; Gly= Glycosides; Sap= Saponins; Ste= Steroids; Res= Resins; Fla= Flavonoids; Phe= Phenols; Tan = Tannins; Pro= Proteins

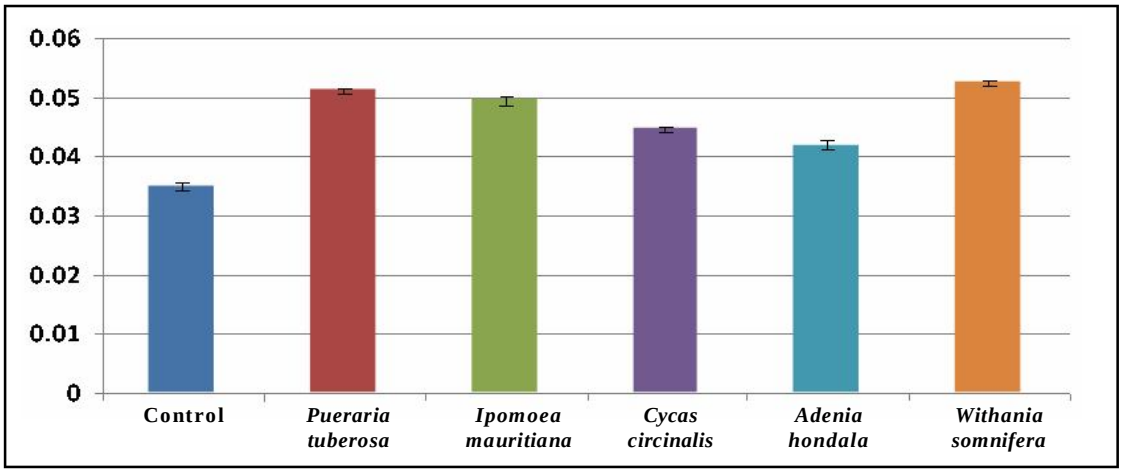


Figure 2: Graphical representation of all test substances, standard and control on granulopectic index (min^{-1})

3.2 Screening of phytoconstituents

All the extracts for different plant species were screened for the presence of the phytochemicals and the results reported that all the plants exhibited some important secondary metabolites, *viz*. carbohydrate, waxes, phenols, alkaloids, glycosides, saponins, phytosterol, flavonoids and gums but absence of tannins. Furthermore, A.h and C.c showed absence of alkaloids and resins (Table 1). The variation of these studies were may be due to the nature of plant sources which further correlated with the earlier studies of Mamoon *et al*. (2013) and Kulkarni *et al*. (2013).

3.3 Maximum tolerance dose (MTD)

The MTD studies were carried out as per OECD guidelines on swiss albino mice of either sex for all the powdered drugs. All the species used as vidari were tested at two different doses, *viz*. 1g/kg and 2 g/kg bw, p.o. Toxic symptoms with the based on various parameters like mortality, tremors, alertness, eye movements and motility, convulsions and mortality were recorded after 24 and 72 h. and resulted that there are no changes on physiological behavior which was found to be safe dose and no toxic symptoms were observed and resulted all the test substances were safe to use for various applications.

3.4 Carbon clearance assay

The test was conducted on wistar rats in 6 groups (n=6) to establish phagocytic activity of reticuloendothelial system after treatment with 250 mg/kg of the powdered mature tubers of P.t, I.m, A.h and stems of C.c as per the standard procedure where control group received normal food and water and dried powder of W.s (250 mg/kg p.o) was used as positive standard and granulopectic index (min^{-1}) were calculated for all the test drugs. The results identified that all the test substances and standard showed significant activity compared to control ($p < 0.001$). The activity of P.t ($p > 0.05$) was found to be very close (98.07%, 0.051/min) to that of positive standard of W.s, (0.053/min), followed by I.m (94.2%, 0.049/min) whereas A.h showed least activity (80.76%, 0.041/min) compared to all other test substances (Figure 2). It was interesting that C.c and A.h also gave better activity as compared to control (0.034/min) even though they are used as adulterants and substitutes in the market. Similarities between phytoconstituents and bioactivity of P.t and I.m makes the latter as good substitute for the former. In fact in Kerala, I.m known as Palmuttukku is used as vidari or kshiravidari (Venkatasubramanian *et al*., 2009).

Bibliographic references revealed components such as polysaccharides, lectins, proteins and peptides present in plants have been shown to stimulate the immune system (Haijto *et al*, 1989). Several immunomodulators like sterols, a group of compounds were identified from plants that have demonstrated promising results in a number of clinical trials. The most studied sterols are beta sitosterol and its glycosides (Patrick and Johan, 1999). Furthermore, Zhao *et al*. (2005) have isolated and purified polysaccharide from *Ipomoea batatas* (Sweet potato) and characterized as (1 → 6)-α-D-glucan that showed *in vivo* immune function of mouse. Administration of an extract from the powdered root of the plant, W.s showed an enhancement in phagocytic activity of peritoneal macrophages when compared to control in mice. These results confirm the immunomodulatory activity of W.s extract, which is a known immunomodulator in indigenous medicine (Davis and Kuttan, 2000). The results of the above surveyed literature confirms that the polysaccharide and sterol are might be the responsible for immunomodulatory activity.

4. Conclusion

Qualitative screening of phytochemicals for all four plant species of vidari indicated the presence of carbohydrates, glycosides, saponins, phytosterols, flavonoids, proteins, phenols, gums and mucilages which are essential secondary metabolites for any activities. Furthermore, alkaloids were found to be present in P.t and I.m whereas resins, fats and tannins were absent in all the vidari species. These variations of the phytoconstituents resulted variant immune response when compared to standard W.s powder and revealed significant activity with P.t, followed by I.m due to similarities of their phytoconstituents but small variation of results due to the effect of cultural conditions. Hence, the present study

demonstrated the potential value of obtaining guidance from traditional knowledge for development of quality standards through pharmacological activities for herbal medicines with the proper authenticated raw herbal drugs.

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Conflict of interest

We declare that we have no conflict of interest.

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