INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
16–0084/2016/18–6–1098–1102
DOI: 10.17957/IJAB/15.0171
http://www.fspublishers.org

Full Length Article

Ethnopharmacological Investigation of *Rhynchosia pseudo-cajan*: A Perennial Shrub for Various Activities

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Abstract

The present study was undertaken to evaluate the antimicrobial, antioxidant and anthelmintic activities of the plant *Rhynchosia pseudo-cajan* Cambess. The crude extracts of powdered plant material were obtained in various polar and nonpolar solvents, viz: petroleum ether, chloroform, methanol and distilled water. Well defined zones of inhibition were recorded indicating that the plants were potent against pathogenic microbes, such as i.e., *Staphylococcus aureus, S. saprophyticus, Escherichia coli* and *Pseudomonas aeruginosa, Aspergillus parasiticus* and *Rhizopus oryzae.* The antioxidant activity of all the plant extracts was studied by DPPH Assay, Total Antioxidant Assay and Total phenolic Assay and the remarkable values comparable with the standard antioxidants were recorded. The *in vitro* anthelmintic activity of these plant extracts was found much stronger than the standard medicine, like Levamisole. Therefore, these plants can be declared as the possible source of antimicrobial, antidiabetic, antioxidant and anthelmintic agents to treat the ailments of man and his domestic animals like sheep, goat etc. © 2016 Friends Science Publishers

Keywords: Antimicrobial; Antioxidant; Minimumm inhibitory concentration

Introduction

Many cultures have lived close to nature (plants), depending on its products for their needs such as food, forage and fodder for their cattle, housing, timber, fuelwood, furniture, bridges, agricultural tools, protection of fields from erosion, household utensils, fences, medicines and above all, for aesthetic, ornamental and religious purposes. These cultures are suffering rapid cultural, social and economic changes. The people have a great understanding of the properties of their local plants because of a particular intuitive and inherited interaction with them. The branch of science that deals with such interaction of people and plants, among the tribal and rural people, for recording their unique knowledge about plant wealth and search for new resources of herbal drugs, edible plants, and other aspect of plants is called Ethnobotany; as a subject has progressed through various stages during the last 100 years (Jain and Mudgal, 1999).

Rhynchosia pseudo-cajan Cambess is an erect ever-green shrub that is common in open grassy hill-sides and in the under growth of chill forests. The branches are striate, grey tomentose. The leaves are pinnately trifoliate and the petioles are 0.5–1.5 inches long, stipules are minute. Apex is usually deltoid. Flowers are 0.5 inches long, yellow in dense axillary racemes, 1–3 inches long pedicels. Calyx is 3 inches long teeth narrow acuminate, the lowest linear-setaceous, exceeding the tube, the others lanceolate. Standard is grey pubescent on the back. Pod is one by 0.3–0.4 inches long, straight, hard, grey-tomentose, and dehiscent, with 1-2 seeds in the upper part (Ali, 1977).

Cano and Amao (2005) analyzed three different *Lactuca sativa* L. varieties (Iceberg, Romaine and Baby head) in order to determine differences in the antioxidant activity, both hydrophilic and lipophilic, and in the total phenolic contents of different leaves (stem, inner, medium and outermost leaves). Romaine showed the highest level of hydrophilic and lipophilic antioxidant activity, and its phenolic content was also higher than that of Iceberg and Baby head.

Villagomez *et al.* (2005) evaluated the total phenolic content and antioxidant activity of extracts from pine species for various plant components, with emphasis on the seed cones. Seed cones from pine species were found to contain relatively high amounts of both total phenolics and antioxidant activity, and there appears to be some correlation of the two measurements. Juvenile cones contained by far the highest phenolic and antioxidant activity, but this high activity appears to be related to seeds retained in some of the samples, possibly due to the presence of proteins or additional antioxidants in the seeds. The phenolic content and antioxidant activity were also measured for other plant components and for a few other

To cite this paper: Aftab, A., Z.U.D. Khan, Z. Yousaf and S. Khalid, 2016. Ethnopharmacological investigation of *Rhynchosia pseudo-cajan*: a perennial shrub for various activities. *Int. J. Agric. Biol.*, 00: 1098–1102

species for comparison. In general, the cones of red and jack pine exhibited the highest antioxidant activity compared to black and southern pines. The general trend for both total phenolics and antioxidant activity was (highest to lowest): juvenile cones>needles>new cones> bark>old cones>wood. Obviously, cones could represent a viable source of antioxidants, especially compared to the wood of species that had comparatively low activity. Collection and extraction of pine cones for antioxidants would be a non-destructive method for procurement of this medicinal aid.

Materials and Methods

The plan of work was designed to evaluate the pharmacological effects of all the plant specimens.

- Collection and preservation of the plant material.
- Solvent Extraction by Maceration Method in the Polar and Non polar solvents e.g. Petroleum ether, Chloroform, Methanol and Double Distilled Water.
- Antimicrobial activity of the plant extracts according to Ortega *et al*. (1996) and Ferreira *et al*. (1996).
- For antioxidant evaluation of the plant extracts DPPH assay by Erasto *et al.* (2004) and Total antioxidant assay by Prieto *et al*. (1999) were applied.
- The total Phenolic Assay was performed according to Makkar *et al*. (1993).
- The Anthelmintic activity was performed according to Singhal (1983).

Plant samples were collected from the GCU Botanic garden during the month of February. All the plant materials were authenticated by Department of Botany. The Voucher specimens of all the plant specimens were submitted to Dr. Sultan Ahmed Herbarium, Department of Botany, GC University, Lahore.

Results

The *R. pseudo-cajan* stem distilled water extract gave maximum yield (8.17 ± 0.06), while stem chloroform extract yielded the minimum amount i.e., 2.59 ± 0.20.

Different polar and non-polar solvents were used for the extraction and thus their antimicrobial aspect and different values of inhibitory action against the microbes in mm were observed. In most of the cases the fungal species were strongly affected. Moreover, the water extracts in majority of the cases showed the maximum inhibitory values against the microorganisms used.

In case of *R. pseudo-cajan*, leaf distilled water extract indicated the maximum inhibitory zone/value (54 ± 0.24^{a}) against *Staphylococcus saprophyticus* and stem petroleum ether extract showed the minimum zone of inhibition (7 ± 0.21^{a}) against *E. coli* among the bacterial strains. For fungi the maximum zone of inhibition was observed in leaf chloroform extract (44 ± 0.527^{a}) against *Aspergillus parasiticus* and stem petroleum ether extract resulted in the lowest value (7 ± 1^{b}) as indicated in Figs. 1–12.

The absorption values at 517nm and %age DPPH values of all the extracts were recorded and later on compared with the standard antioxidant chemicals, BHT (Butyl Hydroxy Toluine) and α-Tocopherol. Leaf methanol extract of *R. pseudo-cajan* showed the %age DPPH value closer to that of the BHT with a value 77±2.95, (Fig. 14)

For total antioxidant assay the absorption values in this case were recorded at 695 nm. All the leaf extracts of *R. pseudo-cajan* proved excellent antioxidants as compared with BHT whereas the stem extracts showed their closeness with the α-Tocopherol (Fig. 15).

The results for Total phenolic Assay were noted down by comparing them with the Gallic acid equivalent and the resultant values were recorded in the form of µg/g of Gallic acid. As the value increases, it means the quantity of phenol is increasing. The whole procedure was carried out on three sample replicates and the mean of these values was being recorded in association with the standard deviation among the replicates.

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As far as the values of *R. pseudo-cajan* were concerned leaf chloroform extract showed the maximum value, 485 ± 12.7 and stem water extract showed the lowest value meaning thereby the lesser phenolic contents (Fig. 16a and b).

The Anthelmintic activity was basically concerned with the effect of plant extracts on the survival of worms (*Haemonchus contortus*) and the worms showed variable survival duration in the extracts depending upon the strength of the extracts. These values were further compared with the control (PBS+DMSO) and the standard medicine Levamisole (Fig. 17–18).

Discussion

The use of herbs and medicinal plants as the first medicines is a universal phenomenon. Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plants for their therapeutic properties. All drugs of the past were the chemical substances with a particular therapeutic action extracted from plants. Thus, medicinal plants may be defined as any plant that can be put to culinary or medicinal use.

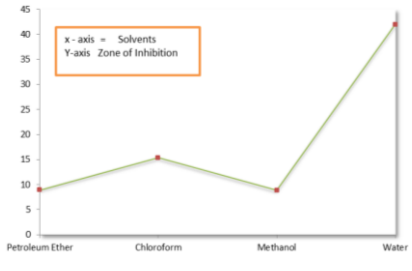

Fig. 1: Zone of Inhibitipn (mm) produced by Leaf extracts against *S. aureus*

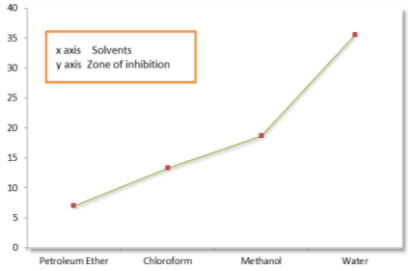

Fig. 2: Zone of Inhibition (mm) produced by Stem extracts against *S. aureus*

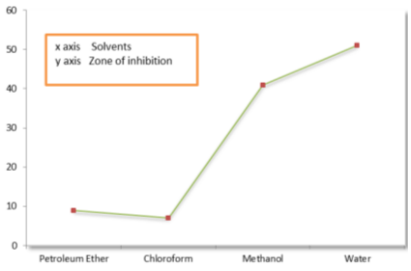

Fig. 3: Fig. 3 Zone of Inhibition (mm) produced by Leaf extracts against *S. saprophyticus*

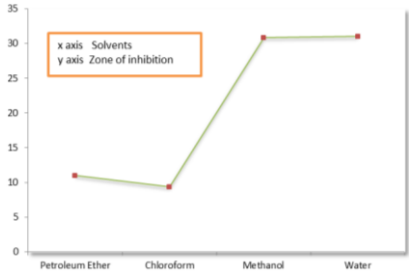

Fig. 4: Zone of Inhibition (mm) produced by Stem extracts against *S. saprophyticus*

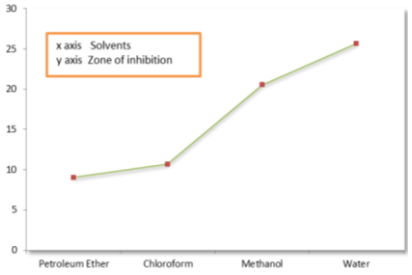

Fig. 5: Zone of Inhibition (mm) produced by Leaf extracts against *E. coli*

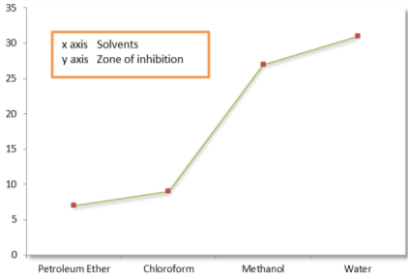

Fig. 6: Zone of Inhibition (mm) produced by Stem extracts against *E. coli*

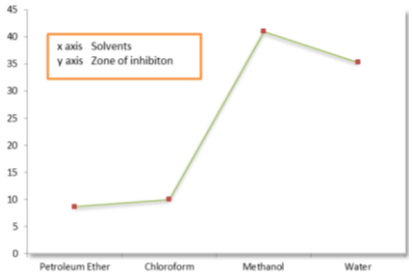

Fig. 7: Zone of Inhibition (mm) produced by Leaf extracts against *P. aeruginosa*

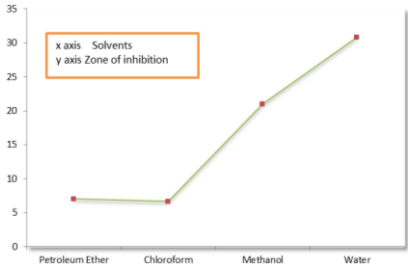

Fig. 8: Zone of Inhibition (mm) produced by Stem extracts against *P. aeruginosa*

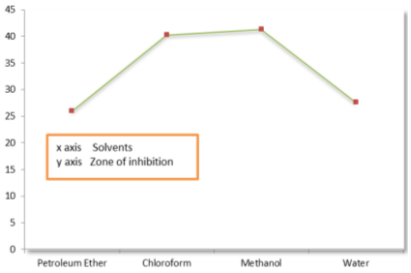

Fig. 9: Zone of Inhibition (mm) produced by Leaf extracts against *A. parasiticus*

The results indicated that all the plants and their portions are antimicrobial in nature; some of them showed very highly antimicrobial against bacteria and fungi, while some of them were comparatively less antimicrobial in nature. A variety of standard antimicrobial discs were run to compare the zones of inhibition against bacteria like *S. aureus, S. saprophyticus, E. coli* and *Pseudomonas aeruginosa,* and fungi like *A. parasiticus* and *Rhizopus oryzae.*

R. pseudo-cajan leaf distilled water extract showed the maximum inhibitory value (54 ± 0.24^{a}) against *S. saprophyticus,* as the polar compounds are thought to be responsible for the high antimicrobial activity that's why the aqueous extracts in most of the cases showed high antimicrobial value. Both aqueous and solvent extracts of various plants have antibacterial capacity and have been used very effectively against different types of the bacteria as reported earlier (Ibrahim *et al.*, 2009).

Funatogawa *et al.* (2004) isolated the terpenoids and phenols from the medicinal plants and investigated their antibacterial potential. The plants used in the present investigation can be considered medicinal in nature due to the significant results showing high antimicrobial potential. A plant found pharmacologically important in the folk and traditional remedies, must be evaluated through studies.

The antioxidant activity of some of the locally found and ethnobotanically important plant was evaluated into different testing systems. The results revealed that plants had significant free radical scavenging activity. The free radical scavenging activity might be one of the mechanisms by which the plant extracts exhibited high antioxidant activity. Hence the present study provided a strong evidence for their use in food industry and medicine. Bajpai *et al.* (2005)

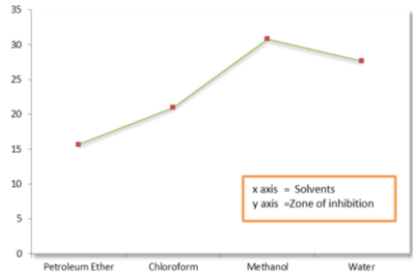

Fig. 10: Zone of Inhibition (mm) produced by Stem extracts against *A. parasiticus*

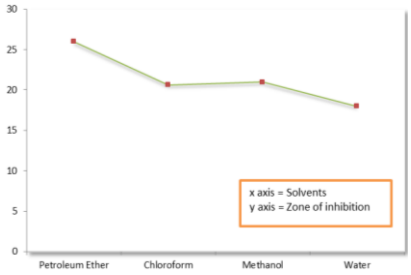

Fig. 11: Zone of Inhibition (mm) produced by Leaf extracts against *R. oryzae*

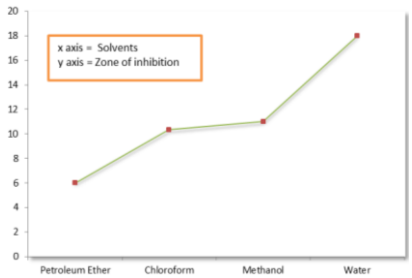

Fig. 12: Zone of Inhibition (mm) produced by Stem extracts against *R. oryzae*

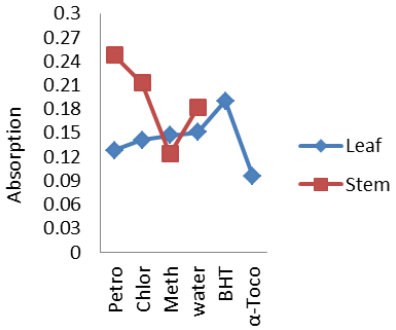

Fig. 13: Antioxidant activity of various extracts of *R. pseudo-cajan* in different solvents through DPPH Assay

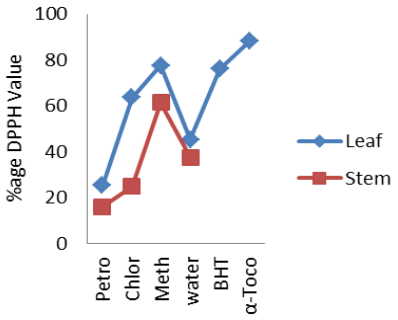

Fig. 14: Percentage DPPH values of various extracts in different solvents

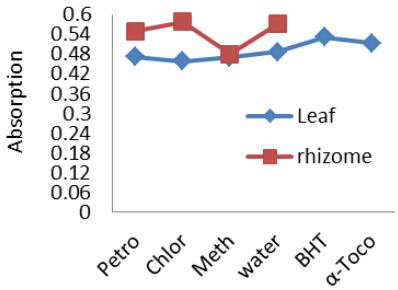

Fig. 15: Antioxidant activity of various extracts of *R. pseudo-cajan* in different Solvents through Total Antioxidant Assay

identified different plant sources as good antioxidants because of the presence of their total phenolic contents and high percentage scavenging activity.

As the extracts of *Ficus palmata* are concerned leaf distilled water extract and stem petroleum ether extracts are closer to BHT with values 74.1 ± 1.61 and 78.01 ± 1.59, respectively. And leaf methanol extracts is closer to α-Tocopherol with the value of 82.8 ± 2.08. Such effectiveness have earlier been reported by Kardosova and Machova (2006).

All the above mentioned extracts were very good antioxidants, comparable with the standard BHT and α-Tocopherol because of the strongest antioxidant activity that's why these plants are considered to be important medicinal plants in the folk and traditional herbal medication system.

As Montoro *et al.* (2005) evaluated varieties of medicinal plants, known in traditional medicine and found high antioxidant activity by different assays. In the same way the activity of these plant extracts was also evaluated by total antioxidant assay and the some of the extracts proved very good antioxidants as comparable with the standard chemicals e.g., All the stem extracts of *R. pseudo-cajan* proved excellent antioxidants as compared with α-Tocopherol while the leaf extracts were comparable with BHT.

In different studies it has been noted that naturally occurring antioxidant compounds from the plant sources have been identified as free radicals or active oxygen scavengers. Hence interest has been considerably increased to exploit natural resources having antioxidant activity to replace synthetic antioxidants, which are being restricted due to their side effects.

As well as the values of *R. pseudo-cajan* are concerned leaf chloroform extract showed the maximum value 485 ± 12 as reported by Stanojenic *et al.* (2009) in which they determined the antioxidant activity and the phenolic contents and the high values of phenols showed that these plants containing specific compounds are strongly active antioxidants.

It is also emphasized that the herbal remedies used in the folk medicines provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help overcome the growing problems. This activity was basically concerned with the effect of plant extracts on the survival of *H. contortus* and the worms showed variable survival duration in the extracts depending upon the strength of the extracts. These values were further compared with the control (PBS+DMSO) and the standard medicine Levamisole.

In the leaf extracts of *R. pseudo-cajan*, chloroform and methanol extracts were stronger than others and even

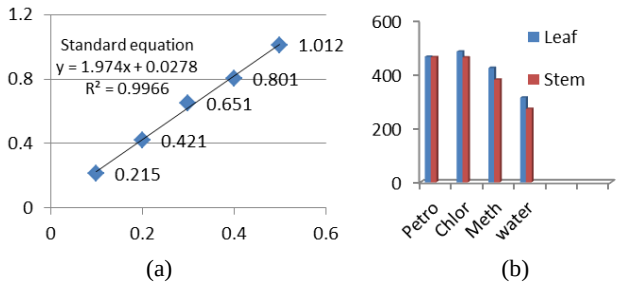

Fig. 16a, b: Antioxidant activity of various extracts of *R. pseudo-cajan* through Total Phenolic Assay

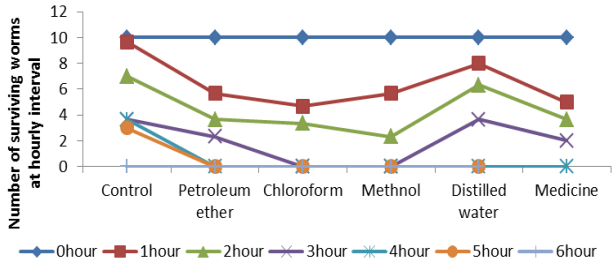

Fig. 17: *In vitro* comparison of anthelmintic activity of different treatments: leaf extracts of *R. pseudo-cajan* in various solvents, standard drug (Levamisole) and control (PBS+DMSO)

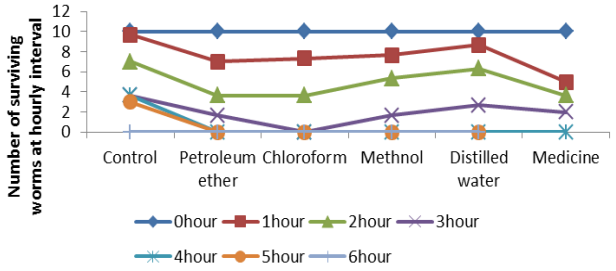

Fig. 18: *In vitro* comparison of anthelmintic activity of different treatments: stem extracts of *R. pseudo-cajan* in various solvents, standard drug (Levamisole) control (PBS+DMSO)

from the standard medicine medicine in killing the worms. Stem chloroform extracts of *R. pseudo-cajan* was the strongest one among all the extracts as the worms survived for 2 h. was also stronger and active than standard medicine. All other extracts showed almost duration of 4 h just equal to standard.

Conclusion

From the entire experimental work it is being concluded that the plant proved very much active as antimicrobial, antioxidant and as well as anthelmintic agent as all the results were being compared with the commercially available standards.

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(Received 17 February 2016; Accepted 16 March 2016)