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PHYTOCHEMICAL SCREENING, ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF PENTANISIA PRUNELLOIDES FROM THE EASTERN CAPE PROVINCE, SOUTH AFRICA

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Abstract

Background *Pentanisia prunelloides* is a medicinal plant widely used to remedy various ailments including infections, fever and rheumatism in Eastern Cape Province of South Africa. There is scanty report on the phytochemical and biological properties of the plant; hence various solvent extracts of the dried plant materials were phytochemically screened, and its aqueous extract evaluated for acute toxicity effect, analgesic and antiinflammatory properties in rodents.
Methods and Materials: Different extracts of both leaf and rhizome were obtained separately with ethanol, methanol and water. Portions of the filtrate were used for qualitative screening of secondary metabolites and remaining portions were concentrated and dried. Dried grounded leaf and rhizome of the plant were also used for quantitative screening for some major components. The aqueous extract of the leaf and rhizome were used for acute toxicity (LD_{50}) test, antiinflammatory and analgesic activities in rodents.
Results: The qualitative phytochemical screening showed the presence of several phytoconstituents with saponins, flavonoids and alkaloids constituting highest constituents in the leaf and rhizome. The LD_{50} of the aqueous extracts (from leaf or rhizome) was found to be ≥5000 mg/kg orally. The leaf and rhizome aqueous extract (250-500 mg/kg) significantly ($p<0.01$) reduced egg albumin-induced paw oedema and paw licking in mice induced by formalin, signifying antinociceptive and antiinflammatory activities respectively.
Conclusion It is concluded that the leaf and rhizome of *P. prunelloides* are rich in various phytochemicals which could be associated with their medicinal uses. The aqueous leaf and rhizome extracts are similarly non-toxic orally, showed antiinflammatory and analgesic potentials thus rationalizing its use in folkloric medicine.

Key words: Rubiaceae, secondary metabolites, oral acute toxicity, analgesic, anti-inflammatory

Introduction

Plants were the primary medicine in the world from time immemorial and they are still used in everyday healthcare needs of majority of people in rural communities (Ndlovu, 2007). In almost all the towns and cities in South Africa, traditional medicines are also available widely and sometimes they can be sourced in specially designated markets known as “muthi” markets and traditional specialists’ shops (Steenkamp, 2003). Orthodox medicine and some scientists believed that the methods of traditional knowledge and practices are primitive and backward (Conserve Africa, 2002). These plant-based medicines are believed to be less toxic and have lesser adverse effects than synthetic drugs and therefore assumed to be safe (De Wet et al., 2005). Knowledge of the chemical constituents of plant is useful in the discovery of therapeutic agent and sources of economic materials such as oil and gum. Important bioactive constituents of these plants include alkaloids, tannins, flavonoids etc. (Satapathy et al., 2009).
Pentanisia prunelloides (Kotzeh ex Eckl & Zeyh.) Walp. (*Rubiaceae*) features as a prominent medicinal plant for treating ailments associated with inflammation, infection, muscular pains, haemorrhoids and antidote to snake bite in Zulu traditional medicine (Hutchings et al., 1996). The plant is reported to be effective in stomach upsets and toothache (Adeniji et al., 2000). Boiled grated dried bulb is usually taken orally to stop vomiting and diarrhoea in children (Bisi-Johnson et al., 2010). Anti-microbial, cytotoxicity and cyclooxygenase-1 enzyme inhibitory activity of the plant has been reported (Jager et al., 1996). Root and leaf extracts of *P. prunelloides* inhibit COX-1 and the viral replication of the influenza virus and further investigation of the plant led to the isolation of palmitic acid as the major anti-microbial agent (Yff et al., 2002; Hashem at el., 1999); (−)-epicatechin was shown to be effective in the treatment diarrhoea (Pretorius et al., 2003).
Phytochemical screening of *P. prunelloides* indicate high concentration of alcohol precipitable solids (0.7–7.0%) and amino acids, while sucrose and (−)-epicatechin have been isolated (Ndlovu, 2007). Palmitic acid was identified as a major compound in *P. Prunelloides* (Yff et al., 2002), diosgenin and oleanolic acid have also been isolated from the rhizome extract of *P. prunelloides* (Mpofu et al., 2014). Cytotoxicity and anti-inflammatory activities of *P. prunelloides* extract has been ascribed to the presence of flavonoids and saponins (Mpofu et al., 2013). Analgesic properties of *P. prunelloides* have been reported (Mpofu et al., 2014). Considering the widespread use of this plant in the Eastern Cape Province and the search for new bioactive molecules from natural sources, *P. prunelloides* was investigated. This study therefore focuses on the preliminary phytochemical screening of the leaf and rhizome of the plant, determined the acute toxicity profile and evaluated their aqueous extracts for analgesic and antiinflammatory

activities in rodents. The outcome of this study is expected to provide new data on the phyto-constituents, safety profile and biological activities of the plant.

Materials and Methods

Plant collection

Fresh leaves and rhizomes of *P. prunelloides* were purchased from Miss. N. Rhadebe at Mthatha "Muthi" Market on the 10th April 2015. The plant was identified by Dr K.L. Immelman, Botany Unit, Department of Biological and Environmental Sciences, Walter Sisulu University, Mthatha and a voucher specimen was deposited in the herbarium with reference number: **MGM 001** issued.

Preparation of the Plant Materials

The rhizomes and leaves were separated, cut into small pieces and dried at room temperature for two weeks before grounded into coarse powder. Aqueous (Aq), methanolic (MeOH) and ethanolic (EtOH) extracts were obtained by soaking the plant materials in large 5 l flaks for 48 h.

Phytochemical screening

Qualitative phytochemical screening of the aqueous, methanolic and ethanolic extracts of *Pentanisia prunelloides* leaf and rhizome was carried out to test for the presence of some phytochemicals including: tannins, flavonoids, steroids, terpenoids, saponins, alkaloids and glycosides (Harbone, 2001, Ghani, 1998 and Sofowora, 2005), as fully described by Dyayiya et al. (2016).

Quantitative Phytochemical screening

The following secondary metabolites: saponins, tannins, flavonoids and alkaloids were quantitatively estimated (Dyayiya et al., 2016).

Biological studies

Laboratory animals

Mice and rats were purchased from the South African Vaccine Initiative, Johannesburg and kept at the Animal Holding Facility, Zoology Unit, and WSU. Male and female Wistar rats (200-300 g) were randomly selected (n=6) for the anti-inflammatory test. Swiss mice of both sexes (25-35 g) were also selected for the acute toxicity and the analgesic tests. The animals were kept under standard laboratory conditions and had free access to rat chow and water. Food was however withdrawn overnight prior to experiments while water was provided *ad libitum*. Wood shavings were utilized as bedding materials. During this period of acclimatization, animals had free access to rat pellets (EPOL SA). Cages were cleaned and bedding replaced 2 times per week. This study was approved by the Department of Higher Education, WSU and Ethical Clearance Approval obtained, Walter Sisulu University Ethics Committee Ref: DVC (AA&R) DRD/SREC: Reference No: 31, in accordance with the "Principles of Laboratory Animal Care", NIH publication no. 85-23, revised 1985 (Guide for the Care and Use of Laboratory Animals).

Drugs and reagents

Formalin (M&B, UK), aspirin (Sigma Chemical Co., USA), normal saline (ACCSYS, PTY LTD, Johannesburg).

Acute toxicity test of the aqueous dried leaf and rhizome extracts of *P. prunelloides*

Mice were used to determine the acute toxicity profile of the plant's extracts (Lorke, 1983). The procedure was divided into two phases. The first phase was tested at 3 dose levels of 10, 100 and 1000 mg/kg per oral (n=3) for either leaf or rhizome aqueous extract. The second phase was tested at 4 dose levels of 1000, 1600, 2900 and 5000 mg/kg per oral (n=1) for either leaf or rhizome aqueous extract. Mice were allowed free access to water but feed withdrawn overnight before test. The treated mice were observed for signs of toxicity within 30 min and for mortality for upward 24 h. Subsequently, the mice were observed twice a day for mortality for 14 days following treatment. The LD_{50} value was calculated according to the Lorke's method (Lorke, 1983).

Anti-inflammatory test of the aqueous dried leaf and rhizome extracts of *P. prunelloides*

The anti-inflammatory activity of either leaf or rhizome aqueous extract was evaluated on egg albumin-induced rat paw oedema model. Different groups (n=6) of rats were orally pre-treated with normal saline (10 mg/kg), the extracts of the dried leaf or rhizome (250 and 500 mg/kg) and aspirin (100 mg/kg)1 h prior to injection of fresh egg albumin (0.1 ml, 50% v/v) into the left hind-paw of the rat (Boughton et al., 1993). Baseline paw size (mm) was noted and recorded before and after 1, 2, 3 and 4 h post injection of the egg albumin with Vernier Callipers (Joseph et al., 2005). Baseline size was measured before and after 1, 2, 3 and 4 h after injection of egg albumin (Sarika et al., 2012).

Analgesic test of the aqueous dried leaf and rhizome extracts of *P. prunelloides* on formalin test

The method used was extracted from Viana et al. (2000) and Hajhashemi et al. (2003) with little modification. Six groups of mice (n=6) were randomly selected. Group 1 (control) were orally administered 10 ml/kg normal saline, groups 2-3 were orally treated with leaf aqueous extract (500, 1000 mg/kg), groups 4-5 with rhizome aqueous extract (500, 1000 mg/kg) and

group 6 treated with aspirin (100 mg/kg, p.o.) 1 h prior to administration of 0.02 ml of 2.5% formalin into the sub-planter space of the right hind paw. The duration of paw licking was determined 0-5 min (1st Phase or neurogenic phase) and 20-30 min (2nd phase or inflammatory phase) after formalin administration (Hunskaar and Hole, 1997; Yin et al., 2004).

Statistical Analysis

All results were presented as Mean±SEM and further analysed with one way analysis of variance (ANOVA) followed by Dunnett's post hoc test, and values were considered significant at $p<0.05$. GraphPad version 3.0 and GraphPad Prism Version 5 copyright © 2013 by GraphPad Software Inc. USA were used for analyzing the results.

Results and Discussion

Qualitative phytochemical screening

Qualitative test results are presented in Tables 1 and 2; and these results showed the various secondary metabolites found in the various extracts of dried rhizome and leaf of the plant respectively. The results in Table 1 indicate that ethanolic extract of the rhizome contained more secondary metabolites, while aqueous and methanolic extracts did not show presence of alkaloids. Generally, flavonoids, glycosides and steroids were detected at higher concentration when compared to the other secondary metabolites. The leaf extracts appear to contain more phytochemicals than the rhizome extracts. Alkaloids were identified in the ethanol extract of the rhizomes, while in the leaves were found present in ethanol and methanol extracts. In the rhizomes, glycoside, steroids and flavonoids were detected at higher concentration in all the extracts while in the leaves extracts, only glycosides were found to have higher concentration in all the extracts. Both plant parts had the same number of secondary metabolites thus leaves can be used instead of the rhizomes since they showed similarity in their secondary metabolites composition. The method employed here is gravimetric and therefore the results obtained presently are preliminary which will need further phytochemical studies to isolate the individual secondary metabolites present in the plant.

Table 1: Qualitative phytochemical screening of various extracts of *P. prunelloides* rhizome

Phytochemical screened	Aqueous extract	Methanolic extract	Ethanolic extract
Alkaloids	-	-	+
Saponins	++	+	+
Tannins	+	+	++
Flavonoids	++	++	++
Glycosides	++	++	++
Steroids	++	++	++
Terpenoids	+	+	+

Key: not detectable is (-), trace amount is (+), abundant is (++)

Table 2: Qualitative phytochemical screening of various extracts of *P. prunelloides* leaf

Phytochemical	Aqueous extract	Methanolic extract	Ethanolic extract
Alkaloids	-	+	+
Saponins	+	++	+
Tannins	+	+	+
Flavonoids	+	++	+
Glycosides	++	++	++
Steroids	+	+	+
Terpenoids	+	+	+

Key: not detectable is (-), trace amount is (+), abundant is (++)

The results shown in Table 2 indicate that alkaloids were not detected in the aqueous extract, while methanol extract showed high concentration of saponins, flavonoids and glycosides.

Table 3: Quantitative phytochemical screening of the dried leaf and rhizome of *P. prunelloides*

Phytochemical screened	Starting material (g)		Remaining material (g)		Percentage yield	
	Rhizome	Leaf	Rhizome	Leaf	Rhizome	Leaf
Saponins	20.636	20.636	0.130	0.110	0.63	0.53
Tannins	0.506	0.506	Not recovered		Not recovered	

Alkaloids	5.036	5.036	0.110	0.091	2.18	1.81
Flavonoids	10.330	10.330	0.605	0.485	5.90	4.70

Quantitative Phytochemical screening

The results obtained for the quantitative estimation of some secondary metabolites are presented in Table 3. The results show that most abundant compounds found in the leaf and rhizome of *P. prunelloides* were flavonoids, alkaloids and saponins. The high concentration of flavonoids in *P. prunelloides* can be suggested to be responsible for its antioxidant reported activities (Mpofu et al., 2014). The leaves had lower concentrations of these secondary metabolites than the rhizomes. Tannins were not recovered in both plant parts studied.

Acute toxicity effect of the aqueous dried leaf and rhizome extract of *P. prunelloides*

The results of the oral acute toxicity of the aqueous extracts of the leaf and rhizome of *P. prunelloides* showed that there were no mortality at doses up to 5000 mg/kg, hence their LD_{50} was estimated to be ≥5000 mg/kg, p.o. This acute toxicity result obtained for the leaf and rhizome of this plant indicate that they are non-toxic orally (Rodricks, 1992; Lorke, 1983) and therefore support the widespread use of the plant in folk medicine.

A

B

C

Figure 1: *Pentanisia prunelloides*: shoot and rhizome (A), grounded rhizomes (B) and grounded leaves (C)

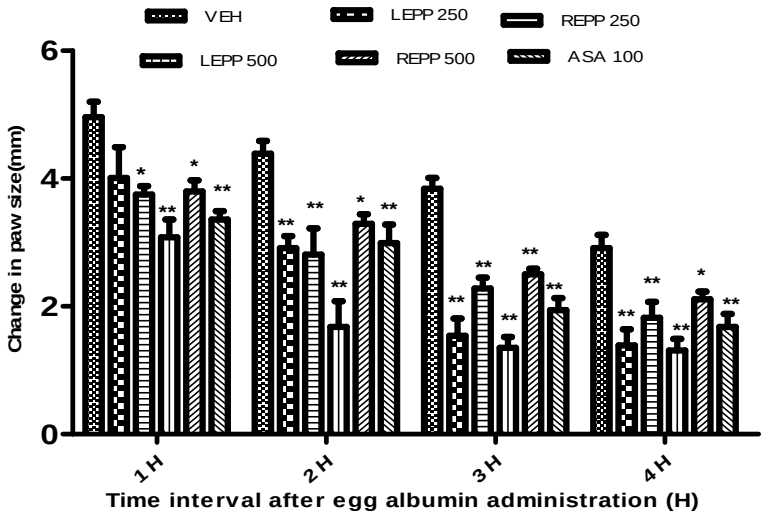

Figure 2: Effect of ethanolic leaf and rhizome extracts of *P. prunelloides* on egg albumin-induced rat paw oedema in rats
VEH, LEPP, REPP and ASA represent vehicle (normal saline/10 ml/kg), aqueous leaf and rhizome extract of *P. prunelloides*, and aspirin respectively. *p<0.05; **p<0.01, significantly lower than vehicle (ANOVA, Dunnett's)

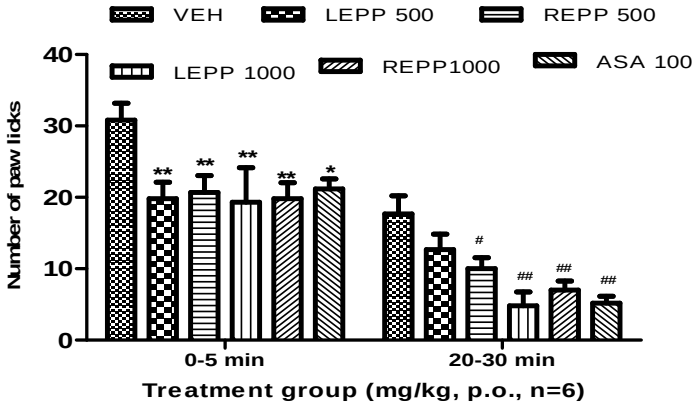

Figure 3: Effect of leaf and rhizome extracts of *P. prunelloides* on formalin-induced paw licking in mice

VEH, LEPP, REPP and ASA represent vehicle (normal saline/10 ml/kg), aqueous leaf and rhizome extract of *P. prunelloides* and aspirin respectively.
*p<0.05; **p<0.01, significantly lower than vehicle at 0-5 min
#p<0.05, ##p<0.01, significantly lower than vehicle at 20-30 min (ANOVA, Dunnett's)

Effect of the aqueous dried leaf and rhizome extracts of *P. prunelloides* on carrageenan-induced paw oedema in rats

The results obtained showed that the leaf extract (250-500 mg/kg) decrease significantly [($p<0.05$-0.01; $F_{(5,30)}$= 5.87, 8.93, 23.56 and 8.07 at 1, 2, 3 and 4 h respectively) the oedema size compared to the vehicle at different time intervals. The rhizome extract caused significant [($p<0.05$-0.01; $F_{(5,30)}$= 5.87, 8.93, 23.56 and 8.07 at 1, 2, 3 and 4 h respectively) reduction in the rat paw oedema throughout the observation period. Comparing the efficacy of the two extracts, it is clearly shown that the rhizome exhibited higher oedema reduction compared to the leaf extract (Figure 2). The standard drug, aspirin caused more and significant ($p<0.01$) reduction in oedema sizes compared to the vehicle and the two extracts. In the first h only the leaf extract at 250 mg/kg failed to reduce inflammation at the higher dose of 500 mg/kg, it significantly ($p<0.05$) reduced inflammation in the first h. All other doses of both rhizome and leaf extracts significantly ($p<0.05$ or $p<0.01$) inhibited carrageenan induced paw inflammation in the rat. The results appeared to be dose- dependent because as the doses were increased the activities were also enhanced. Likewise, the effects observed appeared to be time-dependent for example, the oedema sizes decreases for the negative (vehicle), test agents (extracts) and the positive control group (aspirin) respectively. Flavonoids and steroids which were consistently found abundantly in the extracts could play major roles in mediating the antiinflammatory effects observed here as reported in previous studies (Yerima et al., 2009; Pinheiro et al., 2013). In recent times there has been an upsurge in the use of natural products particularly medicinal plants in the management of pain-related ailments including rheumatism and arthritis (Gao et al., 2009). Narcotics and non-steroidal

antiinflammatory drugs are known for numerous adverse effects including addiction and gastrointestinal disorders (Ong et al., 2007; Almeida et al., 2001). Hence researching into medicinal plants with the aim of discovering novel and potent agents that could circumvent the limitations associated with synthetic agents should be encouraged. Considering the significant antiinflammatory effects demonstrated by the crude extracts of this plant, it is imperative to probe the plant further in order to fully discover its full medicinal potentials.

Effect of the aqueous dried leaf and rhizome extracts of *P. prunelloides* on formalin-induced analgesic test

Figure 3 shows the results for formalin-induced paw licking nociceptive test in mice at two dose levels of 500 and 1000 mg/kg, p.o. The extracts of the leaf and rhizome significantly [($p<0.01$; $F_{(5,30)}=4.46$)] reduced paw lickings in the mice compared to vehicle in the first phase (neurogenic phase) and significantly [($p<0.05$-$p<0.01$; $F_{(5,30)}=7.46$)] reduces the paw- licking in the second phase of the formalin-induced nociception. These results indicate that the two extracts displayed significant analgesic activities in this model. The first phase involved the release of acute phase reactants such as histamine and serotonin while the second phase involves the release of prostanoids with the rate limiting phase being catalysed by cyclooxygenase (Battistini et al., 1994). The extracts of *P. prunelloides* would therefore seem to be acting by inhibiting the release of histamine and related proteins in addition to suppression of cyclooxygenase activity. The first phase is the analgesic phase during acute induction of pains while the second phase represents the chronic manifestation of pains leading to inflammation (Yin et al., 2004). These results also corroborated the anti-inflammatory test results because this phase represents the inflammatory phase (Hunskaar and Hole, 1997). Undoubtedly, the activities of these extracts can be assigned to their constituent secondary metabolites most especially flavonoids, saponins and steroids which were found to be the highest in concentrations in the two extracts. The formalin test is important because it presented two scenarios involved in complex inflammatory processes viz. the early phase which is characterized by immediate response to painful stimulus regarded as the neurogenic-nociceptive as a result of direct stimulation of nociceptors such as bradykinin, serotonin, histamine etc. which are released spontaneously (Chapman and Dickenson, 1992) and in this state, perception of pain is immediate and most pronounced (Khan et al., 2010). The late or delayed phase normally observed after a period of time has been linked to chronic inflammatory phase during which period several inflammatory mediators such as prostaglandins, leukotriene TB4 and other chemotactic agents are simultaneously released (Foster et al.,1986). Agents that are effective in this model would probably found usefulness in varied forms of pain and inflammatory conditions and these present results validate the folkloric uses of this plant species in various medical conditions including headache, fever, rheumatism, back pain and arthritis.

The set of data obtained in this present study showed that *P. prunelloides* has great potentials as a medicinal agent. The oral acute toxicity profile of the two extracts indicates that the plant is very safe for oral administration and provide scientific basis for the long term use of this plant species without serious adverse effects being reported. The extracts obtained from the leaf and rhizome of this plant demonstrated similar and comparative activities, hence the leaf could be used in place of the rhizome in order to preserve the plant. Traditional healers in the Eastern Cape Province of South Africa can be encouraged to harvest the leaf so as to prevent this particular species from going into extinction. The antiinflammatory and antinociceptive potentials of this plant clearly showed that the plant can be useful in managing pain and related musculo-skeletal disorders and can therefore be used to rationalise the application of this plant by the traditional healers from the Eastern Cape of South Africa. Previous *in vitro* tests have reported anti-oxidant and anti-inflammatory activities for this plant species from another province in South Africa (Mpofu et al., 2013), hence, these current results serve to support the reported bioactivities for the plant. It is therefore instructive that this is the first report on the phytochemical screening and *in vivo* biological tests on this particular Eastern Cape species. It is however imperative that further studies be conducted to isolate and characterize the bioactive compounds in this plant as this species show great potentials.

Conclusion

It is concluded from the results obtained in this study that aqueous extracts of the leaf and rhizome of *Pentanisia prunelloides* are rich in several phytoconstituents, are non-toxic orally and exhibited antiinflammatory and antinociceptive activities in rodents.

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