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Phytochemical and Antibacterial Verification of the Ethno-pharmacology of the Root Extracts of *Cissampelos owariensis P. Beauv* and the Implications

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Abstract:
Ethno-pharmacological and phytochemical survey of plants species in the Cissampelos genus of the Menispermaceae family as reported by literature was undertaken. Cissampelos owariensis P. Beauv. was selected as model plant with reference to possible antibacterial potency based on ethno-pharmacology of the alcohol extract of the root. Survey showed abundant ethno-pharmacological activity for the plants in the genus supported by a phytochemistry dominated by alkaloids, terpenoids and steroids. Phytochemical tests on the roots of C. owariensis confirmed the presence of alkaloids, triterpenes, steroids, flavones, and flavonols. Furthermore C. owariensis roots, (100mg/ml in alcohol), showed strong antibacterial activity against six selected strains (Klebsiella spp, Proteus mirabilis, Salmonella typhi, Escherichia coli, Saphylococcus Aureus and Bacillus subtillis), a result that validated some reported ethno-medical utility of the plant, and having been corroborated, throws up fresh posers as the possibility of bothbroad-spectrum potency and even risk factors.

Keywords: *Cissampelos, ethno-pharmacology, trado-medical, bioactive, phytochemistry, antibacterial, chemotherapeutic, quality control, toxicological.*

1. Introduction

1.1. Between Traditional Medicine and Modern Drug Development

Traditional medicine has been described as "the total combination of knowledge and practice, whether explicable or not, used in diagnosing, preventing or eliminating a physical, mental or social disease, which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing" (Sofowora, 1982). Whether as "native medicine" or better as "folk medicine", the defining factor is that in traditional medical practice, health care is provided by using vegetable, animal and mineral matter as well as such relics as fetishes, rituals, juju, incantations, etc. based on socio-cultural and religious belief of the people.

However, nothing projects the statues of the traditional medical practitioner more than his involvement with medicinal plants which are themselves natural reservoirs for physiologically active principles exploited over the years for the trado-medical treatment of various ailments. Modern drug development and the challenge of new drug formulation have for long elevated the traditional medical practitioner to high pharmaceutical relevance since active compounds and other additives from which whole drugs are developed are often natural products present in plants from which traditional remedies are made. "Many modern medicines came about from the isolation and characterization of the active constituents of folklore or traditional remedies"(Danbatta, 1998).

It is pertinent to say that plants and other therapeutic herbs which are plentiful in our environments have made great contributions to traditional medicine, which the advent of modern medicine has not been able to undermine. By such inputs traditional medicine has become a reference library of uses of plants while the traditional medical practitioners are now fundamental consultants to modern drug development (Hostettman *et al.,* 1996). By random sampling of plants for medicinal utility, a researcher could stumble upon some unscreened plants of medical value and by extension new drugs. But such venture can be very herculean considering the diversity of plant families and species. "With an estimated 500,000 plants species, many of whose potential as sources of new drugs remained unexplored, the first embarrassment faced when

screening generally for biological activity is that of selecting which species to be investigated" (Hostettman *et al, 1996*). This is the place where traditional healers come in with their usually wide knowledge of plants and their therapeutic potentials handed down from generations before. They are the intermediaries between the aggressive researcher and the multitude of plants in the universe because they are "important sources of information on the trado-medical uses of plants"(Okor, 1989).

Beyond drawing on trado-medical ideas and ethno-medicine, modern drug development has, however, had to contend with various limitations and pitfalls of trado-medical practice. Such draw- backs include non-standardization of dosage; use of crude unsorted concoctions, decoctions, and unduly bulky powders; non-inclusion of manufacture or expiration dates; non-consideration of toxicological reactions including side effects of adjoining chemical compounds; complete absence of quality control measures since active constituents are largely not known and of course the unverifiable admixture of metaphysical secrets and magic (Gruca, *et al*, 2014). Notwithstanding, plant product have proved useful in the crude and pure forms. Current efforts towards standardization have promoted their acceptance.

Over the years research "has developed from the screening of medicinal plants for bioactive agents to the development of drugs and dosage forms from natural products of merit" (Sofowora, 1982). Active principles or their precursors are extracted from plants and other organisms for characterization and subsequent partial or total synthesis. In addition, new doors are opened for possible structure - activity manipulations leading to either more effective or entirely new drugs.

As it is today, traditional medicine which is of universal spread "has been recognized by the World Health Organization as an essential building block for primary health care, 'Save Plants that Save Lives' is a call to safeguard this heritage' (Okor, 1989).

The use of plant constituents as fundamental clues to drugs development has made very tremendous impact and there is still a very long way to go. It is reported for instance, that over 100 alkaloids have been isolated from the roots of Rauwolfia species including the therapeutically important *reserpine*, *rescinamine*, *deserpidine* and *yohimbine*. Reserpine which is implicated in the sedative properties of the roots also exhibits anti-hypertensive properties and is used in modern medical practice to treat hypertension and mild anxiety. Structural modification of the reserpine structure has led to gain, loss or change of therapeutic activity(Sofowora, 1982).

Another factor that has encouraged modern drug research is the realization that isolated active extracts are in most cases in small quantities inadequate to cater for the increasing needs of humanity and hence the search for synthetic drugs. Furthermore, medicinal plants face threat of extinction from use, civilization, ecological and other environmental factors. Drug assays provide the escape route by almost always ending up in total synthesis of whole drugs in high enough yield to meet man's needs.

Evidence from literature show that in certain cases, the use of pure forms of active principles as against their chemical modification is favoured (Akinniyi and Sultanbawa, 1983). A good example is the story of *quinine* and *Chloroquine*: Quinine has been identified as the active antibacterial constituent of the bark of *Cinchona* species. However, the toxic side effects of the alkaloid such as impaired hearing on prolonged use led to its structural modification to obtain *chloroquine*. But today,"many *chloroquine*-resistant strains (*plasmodium falciparium*or *p. vivax*) now exist; but quinine … is still preferred to treat chloroquine-resistant strains of malaria" (Danbatta, 1998).

The implication of this is that too much might have been inadvertently placed on the shoulder of trado-medical practitioners. Withthousands of phytochemicals from plants having many beneficial biological activities (such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity) being reported as effective with less adverse effects than chemically synthesized drugs (Ingle, Deshmukh, Padole, Dudhare, Moharil,& Khelurkar,2017), doors of abuse and other risk indices may be thrown wide open since issues of standardization are still work in progress.

1.2. Ethnobotany and Ethno-pharmacology of Cissampelos Species from Literature

1.2.1. Cissampelos capensis

Found in the Cape regions of South Africa, the leaf of this plant was used as antidotes to snake bites (Irvine, 1961)

1.2.2. C. mucronata (A. Rich)

The plant is quite common in Senegal, Nigeria, in dry zones of tropical Africa (Olembo *et al.,* 1999). The plant was used for treatment of various ailments: whooping cough (roasted root ash), wounds (roasted shoot ash), snake bite (roasted roots or vegetative parts), ground fresh tops for abdominal/colic pain and irregular menstrual circle (Adjanohoun *et al.,* 1989).

1.2.3. C. owariensis (P. Beauv)

The plant is popular in Cote d' Ivoire, Angola and parts of Nigeria. It is known as Júúdàr ƙásà by Hausas (the same name for C. *mucronata*), Ewe jokoje or *Jokoje* or *Jenjokoo* among Yorubas. In the Okwankwo division of Cross River, it is known as *Kilo-mpape.* It was used variously as purgative; against threatened abortion (ground leaves added to soup), and the leaves with other plants was used against feminine infertility (Adjanohoun *et al.,* 1989).Around Middle Belt Nigeria the shade-dried root is popular as "*egbo paranpupa"*(red medicine for worm) among Yorubas and called *ganga mau*in Hausa. It is used in

admixture with the white counterpart *paran fufu* to combat colic and intestinal parasites as a vermifuge (Abubakar, 2003). It is also reportedly used against headache and fevers (Yusuf, 2003), especially typhoid and malaria fevers, in admixture with other plants e.g. *Aristolochia ringens* (Aristolochiaceae) root and condiments. When ground, the powder is blended with 'pap' or dispersed in tea and drunk "to treat 'sweet breast' in nursing mothers and stomach troubles with special adaptation for the baby's stomach (Said, 2003).

1.2.4. C. pareira (Linn)

The plant is found in tropical and sub-tropical regions. Utility depends on location. Roots were generally used for healing of fresh wounds, boils, burns, insect and snake bites(Hostettman *et al.,* 1996). In tropical areas, the herb was also used to prevent threatened miscarriage and to stop uterine hemorrhages (Lewis and Elvin-Lewis, 1977). In Nepal, the bitter root was reported to be useful as anti-periodic, diuretic, purgative, stomachic, in dyspepsia, diarrhea, dropsy, cough and urinary troubles (e.g cystis), while the leaf was applied externally for itch.

1.3. Phytochemistry of Cissampelos Speciesfrom Literature

1.3.1. Cissampelos glaberima (A. St-Hill).

The tracheal relaxant activity in this plant has been traced to aporphinic alkaloids including trilobine (Carmelio *et al.,* 1999).

1.3.1.1. C: Pareira(Linn)

Parts of *pareira* especially the root were reportedly used for treatment of wounds, boils, burns, snake's bites, to prevent miscarriages and stop uterine hemorrhages. It was also reportedly useful in Nepal as antiperiodic, diuretic, purgative, stomachic, in dyspepsia, diarhoea, dropsy, cough and urinary troubles, while the leaf was applied for itch. A multitude of known and new compounds have equally been isolated from *pareira* (Okogun, 1986). Some are pareirubrine A & B (antileukemic tropoisoquinolinealkaloids), cissampelatlavone, (achalcone-flavone dimer), berberine, cycleanine, isochondodendrine, magnoflorine, nuciferine, tetrandrine andisotetrandrine (both muscle relaxant agents).(Southon and Buckingham, 1989)

1.3.1.2. C. Sympodialis Eichl.

Alkaloids isolated from this plant include roraimine (a bisbenzylisoquinoline alkaloid), liriodenine (a known oxoaporphine alkaloid) - both from the root, and *wariftein* (a bisisoquinoline alkaloid)(Melo *et al.,* 2003)- fromthe leaf. Warifteine has regulatory effect on intracellular Ca^{2+} asa means of controlling spasmolytic activity. It is also present in the rhizomes of *C.ovalifolia*(Southern and Buckingham, 1989).

1.3.1.3. C. Owariensis

Some alkaloids found in the root extract include: bisbenzylisoquinoline, isochrodrondendrinberberine and cycleanine (Southern and Buckingham 1989). Bis (2methoxy ethyl) phthalate and hexahydro1,3-dimethyl(-4-phenyl-1H-azepine-4) carboxylic acid have also been isolated from the root (Efiom, 2010).The ethanol leaf extract of this plant has been reported to contain alkaloids, flavonoids, saponins and tannins(Erhihie *et al.,* 2015).The plant has also investigated for its anti-diabetic activity, where it showed a significant lowering effect in blood glucose level of alloxan monohydrate induced diabetic rats(Ekeanyanwu *et al.,* 2012)

1.4. Statement of the Problem

Phytochemical support and proof of biological activity is key to justify ethno-pharmacological claims on plants parts being currently used in ethno-medicine. Though from literature a phytochemistry that implicated mostly alkaloids, terpenoids and steroids was rife pharmacological claims on our model plant seamed bogus.

Ethno-medically, *C. owariensis* was used as purgative, against threatened abortion and feminine infertility (leaf) (Olembo *et al.,* 1995). The root was used against headache, typhoid, malaria and other fevers, and also as vermifuge among others (Said, 2003; Muazu, 2003). These claims are one too many and tends towards conferring broad spectrum antibiotic potency on the plant. It may be pointed out that typhoid and scarlet fevers, for example, are both bacterial diseases, caused by *Salmonella typhi* and *Streptococcus pyrogenes* respectively. This calls for biological re-evaluation of the plant extract for antibacterial activity to further trail these ethno-medicinal uses. There is need to explore the implication of the result and place it side by side with the ethno-medicinal use and define the next line of challenge to both trado-medical practitioners and modern researchers.

1.5. Objectives

This work seeks to:

1. Compare for concurrence the ethno-pharmacology of *Cissampelos owariensis* (P.Beauv) and other species in the genera with their reported phytochemistry.

2. Subject the root extracts of *C. owariensis* to phytochemical profiling for justification or otherwise of ethno-medical uses.
3. Undertake the biological evaluation of the root extracts of *C. owariensis* root with reference to its possible antibacterial potency.
4. Explore the possible implication of the result on the ethno-medicinal use of the plant part and the future of medicinal plant research.

2. Materials and Methods

2.1. Collection and Preparation of Plant Material
Plant materials (roots) were sourced from Ilorin, Kaduna and Ibadan through the assistance of traditional healers and local dealers. Plant materials so collected were dried in the shade and crushed to powder.

2.2. Extraction of Plant Material

2.2.1. Cold Extraction
About 30g of powdered root of C. *owariensis* was subjected to cold extraction with 1 liter of 45% ethanol. Extraction was carried out overnight, assisted by electronic agitator.

2.2.2. Hot Extraction
About 30g of powdered dried plant root was subjected to continuous counter current extraction with 1 liter of ethanol in a Soxhlet apparatus (quick fix type) for twelve hours.
The extracts were concentrated to dryness by means of a rotary evaporator and stored.

2.3. Phytochemical Screening
Plant extracts were screened for alkaloids, steroids and triterpenes, flavonosides and flavones aglycons, anthracenosides (anthraquinones), coumarine derivatives, saponins, tannins and phlobatannins using standard procedures. Results are presented in Table 1.

2.4. Antibacterial Screening of Crude Extracts
To ensure that the microorganisms were in their active phases, the isolates were inoculated in the nutrient broth and incubated overnight at 37^{o}C. Crude alcohol extracts of the roots of *C. owariensis* were screened for antibacterial activity (*Klebsiella spp, Proteus mirabilis, Salmonella typhi, Escherichia coli, Staphylococcus Aureus and Bacillus subtillis*) using the agar well diffusion method (Singleton, 1981). The sensitivity test agar was employed as medium. Gentamycin at 5ug/ml and 45% ethanol were used as positive and negative controls respectively.

3. Results and Discussion

3.1. Phytochemical Screening of C. owariensis Root
Results of phytochemical screening of *C. owariensis* root in (Table 1) indicated the presence of alkaloids, triterpenes, steroids, flavones and flavones. This result provides some concurrence to its ethno-pharmacological history from literature. Active principles within these families of secondary metabolites might as well be present. From the ethno-pharmacology of the plant implicated chemotherapeutic agents might include antibacterial (bactericidal and bacteristatic) agents, antiseptics, disinfectants, vermifuges, stomachics, antipyretics, anti-infertility agents as well as general tonics.

	Phytochemical	Test	Result
1	Alkaloids	Mayer's reagent test Dragendorffs reagent test	+ve +ve
2	Steroids and Triterpenes	Salkowski's test Liebermann-burchard test	+ve +ve
3	Anthrecenosides or Anthraquinones (emodol aglycons)	Borntrager's Reaction	-ve
4	Coumarins	General	-ve
5	Flavonosides and flavone aglycons	Shibata's test Flavone aglycon	+ve +ve
6.	Saponins	Froting test	-ve
7	Tannins (Gallic and Catechol tannins)	Ferric chloride test Formaldehyde test Phlobatannins	-ve -ve -ve

Table 1: Result of Phytochemical Screening

Key

+ve	Positive
-ve	Negative

3.2. Antibacterial Screening of *C. owariensis*

From the antimicrobial screening result of (Table 2) *C. owariensis* root showed strong potency against six selected strains: Klebisiella spp, *Proteus mirabilis, Salmonella typhi, Escherichia coli, Staphylococcus aureus and Bacillus Subtilis.* The result corroborated the use of the plant material against typhoid and other fevers. Positive antibacterial activity of the plant could therefore mean chemotherapeutic potency against not only diseases attributed to the six selected strains but possibly against more. *Klebisiella spp* is known to be one of the causative agents for cystis. *Salmonella typhi* is implicated in the attack of typhoid fever and food poisoning. *Escherichia coli* is the causative agent for cystis and food poisoning while *Staphylococcus aureus* is responsible for cellulites, conjunctivitis, food poisoning and pneumonia. It may therefore be put forward that *C. owariensis* root could not only have chemotherapeutic potencies against the listed diseases but should be tested further for potency against other bacterial diseases which include: anthrax, botulism, cholera, diphtheria, dysentery, gonorrhea, leprosy, meningitis, syphilis, scarlet fever, tetanus, trachoma, tuberculosis etc.

	BACTERIAL STRAIN	*C. owariensis* 100mg/l	GENTAMYCIN 5ug/l	ETHANOL 45%
1	Klebisiella spp	+	++	-
2	*Proteus mirabilis*	+	++	-
3	*Salmonella typhi*	+	++	-
4	*Escherichia coli*	+	++	-
5	*Staphylococcus aureus*	+	++	-
6	*Bacillus Subtilis*	+	++	-

Table 2: Results from Antibacterial Screening of the Ethanol Extract of C. owariensis Root.

Key

++	Very strong activity
+	Strong activity
-	No activity

3.3. Conclusions, Implications and Recommendations

Phytochemical test carried out on root extracts of *Cissampelos owariensis*P. Beauv showed the presence of alkaloids, triterpenes, steroids, flavonols and flavones which agrees with reports of phytochemical test in other parts of the plant (Erhihie *et al.,* 2015), thus lending credibility to reported abundant ethno-medicinal uses suggesting that the root contain principles having strong chemotherapeutic activity against diseases caused by pathogens like bacteria.

From biological evaluation, the alcohol extracts showed strong potency against *Klebsiella spp, Proteus mirabilis, Salmonella typhi, Escherichia coli, Saphylococcus Aureus and Bacillus subtillis* appears to validate the ethno-medicinal use of the root extract against bacterial diseases like typhoid and other fevers a result that has also been corroborated (Akande*et al*2013).C. owariensis root should therefore be tested further for potency against other bacterial diseases which include: anthrax, botulism, cholera, diphtheria, dysentery, gonorrhea, leprosy, meningitis, syphilis, scarlet fever, tetanus, trachoma, tuberculosis etc.

In line with the disposition that "Many of our 'toys' have toxic potential" (Tommy, 2004), there is need to assess the risk factors of greater surge in the use of the root extracts of *C. owariensis* as a potent ethno-medicine following affirmation of wider range of potencies. One way of doing this is by undertaking elemental analysis of the root ash so as to place the utility of the ethno medicine side by side with its risk indices.

Finally, the isolation and characterization of bioactive and novel compounds from the potent plant part (Zumbes et al, 2016) will help speed up work towards standardization of the herbal remedy as well as widen the scope of risk assessment to cover of adjoining chemical compounds.

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