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RESEARCH ARTICLE

Povidone-Iodine bonded with Natural Herb of *Azadirachta indica* (Neem) to increase the Antimicrobial Activity

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ABSTRACT:
Povidone iodine has long been used by surgeons as a preoperative antiseptic, researchers have been looking at the agent for treating viral conjunctivitis as well as other ocular diseases. The antimicrobial skin-preparation formula includes povidone-iodine and extract of *Azadirachta indica* (neem). The microbicidal action spectrum of povidone iodine (PI) is broad, even after short exposure times. Further, unlike local antibiotics and other antiseptic substances, no resistance seems to develop. The high degree of bactericidal efficiency with respect to highly resistant gram-positive pathogenic micro-organisms, such as methicillin-resistant Staphylococcus aureus (MRSA) and Enterococcus strains, has made the agent particularly useful and significant for hospital hygiene. The antibacterial activity of *Azadirachta indica* (neem) performed by using agar well diffusion method showed a result showcasing an effective limit when as opposed pseudomonas vulgaris for ethanol being used as solvent for extract. In *Azadirachta indica* (neem) leaves extract terminate most propitious source. Iodine and its antibacterial properties have been used for the prevention or management of wound infections for over 150 years. However, the use of solutions (tincture) of iodine has been replaced by the widespread use of povidone-iodine, a water-soluble compound, which is a combination of molecular iodine and polyvinyl pyrrolidone. The resultant broad spectrum of antimicrobial activity is well documented and its efficacy, particularly in relation to resistant micro-organisms.

KEYWORDS: Polyvinylpyrrolidone, microorganism, disinfectants.

INTRODUCTION:
Antiseptics are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. Antiseptics are generally distinguished from antibiotics by the latter´s ability to be transported through the lymphatic system to destroy bacteria within the body, and from disinfectants, which destroy microorganism found on nonliving objects.

The new antimicrobial skin preparations useable to disinfect a surgical site for surgery. The antimicrobial skin-preparation formula includes povidone-iodine and extract of *Azadirachta indica* (neem).

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The new pre-operative skin-preparation quickly and effectively kills microorganisms when applied to the surgical site. The skin-preparation continues to effectively inhibit microorganism growth in the applied area over an extended period of time. The role of the newly developed formulations of povidone-iodine preparations, its antimicrobial activity, the possibility of impairing the wound healing process, the role of povidone-iodine in the problem of growing resistance against antibiotics and antiseptics.

Poly vinyl pyrrolidone–iodine (Povidone-iodine) is a widely used antiseptic introduced by Shelanski and Shelanski (1) in 1956. The preparations of poly vinyl pyrrolidone-iodine commercially available are povidone-iodine solution, scrub, ointment, and foam; of these, the solution is most commonly used. The 10% povidone-iodine solution contains 90%water, 8.5% polyvinylpyrrolidone, 1% available iodine and iodide (2). poly vinyl pyrrolidone is a similar to dextran. The

molecular weight of the poly vinyl pyrrolidone utilized in poly vinyl pyrrolidone-iodine solutions ranges from 10 to 40000 daltons (3). Micro-organism on the skin can be transient or resident. Transient microorganisms lie on the surface of the skin, while resident microorganisms are found at deeper site of the skin (4-7).

Some 30 years after the synthesis of povidone-iodine, a paradox in the behaviour of 10% solutions was reported: its antibacterial action increased with the degree of dilution (low concentrations, i.e. 0.1% to 1%, were more rapidly bactericidal than a full-strength, i.e. 10%, solution) (8). One hypothesis is that the concentration of "free" iodine significantly contributes to the bactericidal activity of povidone-iodine solution: dilution of povidone iodine results in weakening of the iodine linkage to the carrier polymer with a concomitant increase in the amount of elemental (free) iodine in solution (9,10).

Poly vinyl-pyrrolidone-iodine newer vehicles include PVP-I Solution, PVP-I Ointment, PVP-I Cream (11) and PVP-I Gel Alcohol (12). Calfee found no significant difference in skin disinfection among 10% PVP-I, 70% isopropyl alcohol, tincture of iodine and PVP-I with 70% ethyl alcohol (Persist), although there was some evidence suggesting greater efficacy among the alcohol-containing antiseptics (13). This was confirmed in another study by Arata et al. (14). Wiping aqueous PVP-I 10% off after 30 seconds of application did not show a significant difference in the reduction from baseline counts of skin flora at 5, 30, 60 and 120 min.

Azadirachta indica (Meliaceae) commonly known as neem is native of India and naturalized in most of tropical and subtropical countries is of great medicinal value and distributed wide spread in the world. Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin (15,16) Jacobson et al., 1990. It is used as anticancer agent and it has hepato-renal protective activity and hypolipidemic effects ((C) Fitoterapia part I and part II). Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents (17-20) Mahesh and Satish et al., 2008). Almost every part of the tree is bitter and finds application in indigenous medicine.

EXPERIMENTAL METHODS:

Preparation of PVP-i:

Weight around 2g of citric acid poured into 2 neck round bottomed flask. Then 40g of Iso propyl alcohol added slowly with constant stirring. Then 4g of Iodine is added. Above the mixture stirring for 12 hrs. The prepared paste is dried in vacuum oven for 1 hr at pressure 50 and temperature also 50ºc. Then the crude sample is crushed into fine powder and sieved using 1200 mesh cloth**.**

PREPARATION OF LEAF EXTRACT:

20-30grams of fresh leaves were boiled with 200mL of solvent for 1 hour. The extract was filtered using Whatmann filter paper No. 1 and then concentrated in vacuum at 40°-50°C using a rotary evaporator. Evaporation of solvent in the rotary evaporator affords a crude extract of the soluble components and these extracts were subjected to the qualitative phytochemical analysis and antibacterial

RESULT AND DISCUSSION:

The findings of the preliminary Phytochemical investigations and the results of antibacterial activity were depicted in the respective Tables. The preliminary phytochemical tests performed were of qualitative type and from the phytochemical investigations it was observed that alkaloids, tannins, flavonoids, terpenoids, saponins Glycoside and compounds reducing were present in the extracts. The ethanol, chloroform and aqueous extract showed considerable activity against *Salmonella typhii*, The ethanol extract was more active than the standard against Salmonella typhii. Previous study conducted by (Ben Gueddeur et al., 2002) (21) suggests that the essential oil of O. majorana posses antibacterial activity.

The work conducted by (Farooqi and Sreeramu, 2004) (22) reveals that the leaves of marjoram have antibacterial activity against Escherichia Coli, *Pseudomonas aeroginosa*, *Staphylococcus aureus* and *Salmonella typhii*. similarly antibacterial activity of ethanol, chloroform and water extract of Marrubium vulgare, was further assessed against, Salmonella typhii, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa, were recorded (Al-Bakriet al., 2006) (23). The presence of these phytochemical components may be responsible for the observed antimicrobial activity of the plant leaf extract. This findings conforms to there port of (Anyanwu and Dawet, 2005) (24) in which similar constituents was found to exhibits anti protozoal and antibacterial activities. Flavonoid has also been reported to have greater potential benefit to human Health (Jouad et al., 2001 (25).

Imaran Khan et al., 2010 (26) studied that phytochemical analysis of *Azadirachta indica* leaves by using different solvent such as Petroleum ether, chloroform, methanol show the presence of triterpenes, glycosides and fatty acids. Other phytochemicals studied in this analysis were absent in all extract of leaves. Antibacterial activity of *Azadirachta indica* was analyzed by previous workers showed that the chloroform extract of leaves possess

significant activity, than petroleum ether and methanol extracts. Early studies proved ethanol as the most efficient solvent for extracting broad spectrum of antibacterial compounds from plants. Himal Paudel Chhetri et al., 2008 (27) reported that the ethanolic extract of *Azadirachta indica* whole plant shows presence of flavonoids and tannins only. Similarly the extract of *Azadirachta indica* is active against E. coli followed by Staphylococcus aureus. Earlier observation done by (Srinivasan et al. 2001) (28) also showed the antifungal and antibacterial activity of A. Indica.

UV SPECTRAL STUDIES:

The fine structure of the UV spectra of PVP in water at relatively high concentration is entirely reproducible. Very few polymers exhibit fine structure in UV spectra unless the chain unit is of sufficiently complicated structure on dilution of the PVP solution with water the spectrum is shifted to shorter wavelength and below a weight concentration of about 0.002% and maximum as apparently shifted to a wavelength below 210mμ. We have followed the polymerisation of PVP as a function of time by absorbing the decrease in absorption at 235mμ.The polymerisation was carried adding to a 20% of aqueous solution of PVP-I. It was found that the weight of polymer used is close to that calculated from the decrease in absorption. Molecular iodine as an absorption in the near UV which is considerably ultered in the presence of PVP. The alternation in absorption of molecular iodine at 290mμ as a function of PVP concentration is illustrated in absorption at 290mμ of the solution to that of the same concentration of iodine alone is determined. PVP-I Shows high absorbance at 300nm and slight absorbance at 365nm, PVP-I+NEEM there is only slight absorbance at 290nm and 360nm shows less absorbance compared with PVP and NEEM there is only slight absorbance at 290nm and 360nm shows less absorbance compared with PVP-I+NEEM.

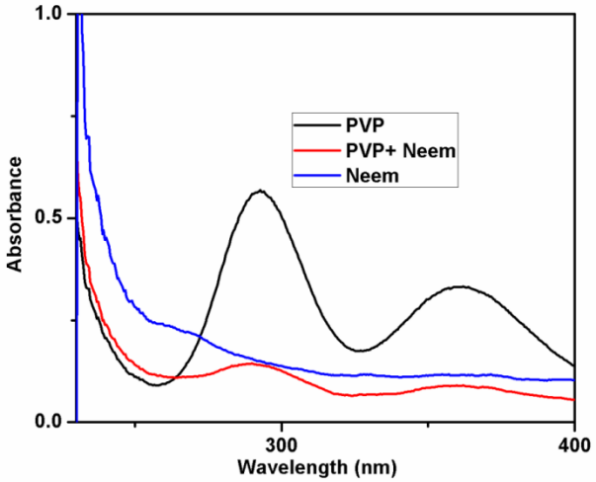

Fig.1 UV Spectra

IR SPECTRA FOR PVP-I:

The iodine loss of aqueous solutions of the polyvinylpyrrolidone-iodine was 18-20 % or 2-4% and was independent of the preparation method. Apparently even proportion is introduced or formed during heating readily found the pyrrolidone group carbonyl group needed for complexation due to the large molar excess of polypyrrolidone monomer units. Even without detailed knowledge of the origin of the adsorption, it is apparent that the IR spectra of the model substances agree with the polyvinyl pyrollidone spectrum. Therefore the frequency and intensity of the vibration of the CN , NC=O and carbonyl group and the ring are largely independent of the alkyl side chain.

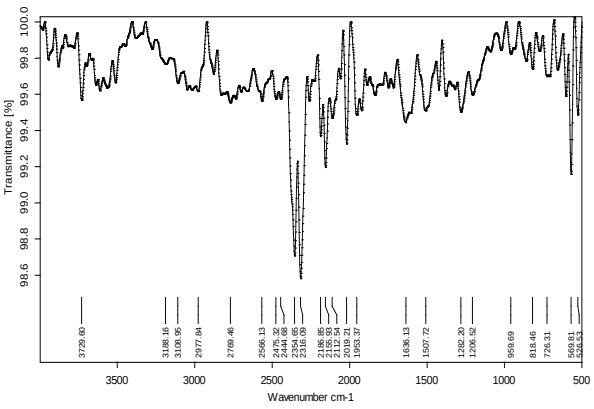

Fig.2 IR Spectra for PVP-I

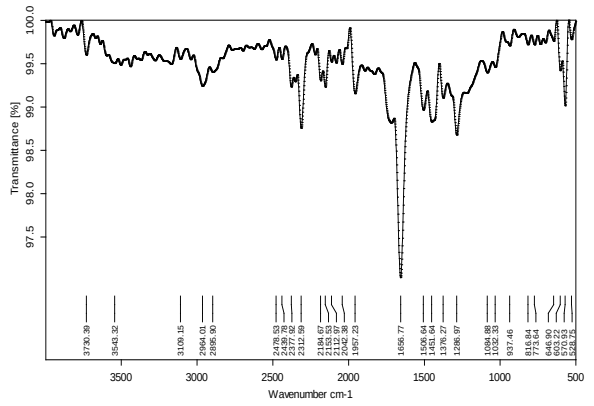

Fig.3 IR Spectra for PVP-I with Neem

The bonds in the complexes of the model substances and in the corresponding polyvinylpyrrolidone adduct must be least very similar. This view may be confirmed by comparing IR spectra of model substances with those of polyvinylpyrrolidone. Hydrogen iodide shows peak at 567 to 815 cm^{-1} NC=O shows peak at 1653 cm^{-1}, five membered ring shows peak at 1745 cm^{-1} C-H bending shows peak at 1375 to 1464 cm^{-1} and C-H stretching shows peak at 2953 cm^{-1} and OH shows peak at 3443 cm^{-1}

PVP-I with Neem:
Hydrogen iodide shows peak at 567cm^{-1} to 815 cm^{-1} NC=O shows peak at 1653, five membered ring is absent, C-H bending shows peak at 1375cm^{-1} to 1464 cm^{-1} and C-H stretching shows peak at 295cm^{-1} and OH shows peak at 3443cm^{-1}. From the graph we came to know that in the binding of PVP-I with *Azadirachta indica* all the functional group of PVP-I is existing in the mixture.

PHYTOCHEMICAL ANALYSIS:
The extracts were analyzed by the following procedures to test for the presence of the alkaloids, saponins, tannins, Terpenoids, flavonoids, glycosides, volatile oils and reducing sugars

SAPONIN:
Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

TANNIN:
To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue color is observed for gallic tannins and green color indicates for catecholic tannins

REDUCING SUGAR:
To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugar

ALKALOIDS:
2ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicate the presence of alkaloids.

FLAVONOIDS:
4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones

ETHANOL EXTRACT:
Azadirachta indica leaves (100 g) were ground into fine powder ((M) Himal Pauel Chhetri et al., 2008) (24) using a stainless-steel grinder, and deep in100% ethanol (200 mL) for overnight. The ethanol fraction was separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 02). The filtered extract was concentrated by a rotary film evaporator.

Table-1: Phytochemical Analysis of Leaves of *Azadirachta indica* leaf.

Solvents used in extraction	Alkaloids	Reducing sugar	Flavonoid	Saponin	Tannin	Volatile oil	Glycoside	Terpenoids
Acetone	-	+	+	+	-	-	-	-
Methanol	-	+	+	+	-	-	-	+
Ethanol	+	+	+	+	+	-	-	-

The phytochemical analysis of plant extracts using Acetone, Ethanol and Methanol was showed in Table - 1. From the phytochemical analysis catecholic reducing sugar were found in *Azadirachta indica* in the solvents such as Acetone, Ethanol and Methanol. The Ethanol extract of *Azadirachta indica* showed the presence of flavonoids, saponins, tannin, reducing sugar were found in presence of Ethanol extract. Reducing sugar, glycosides were observed only in Acetone extract of *Azadirachta indica*. In plant all extracts found glycosides except in Ethanol extract of *Azadirachta indica*. Saponin were observed in the Acetone and Ethanol extract of *Azadirachta indica*. Terpenoids were observed only Methanol extract of *Azadirachta indica*. The Acetone, Ethanol and Methanol all extract of *Azadirachta indica* showed the absences of alkaloid and volatile oil.

Determination of anti bacterial activity:
The antibacterial activity of the leaf extracts was determined using agar well diffusion method by following the known procedure. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 18 hours and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition.

Table-2: Antibacterial activity of Acetone, Ethanol, Methanol Extract of *Azadirachta indica* medicinal plants against human pathogens

Plants	Extracts	*Escherichia Coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Salmonella typhi*
		Zone of inhibition (mm)			
Azadirachta indica	Acetone	17	15	18	16
	Methanol	16	17	12	20
	Ethanol	24	20	19	30

Table-2 showed the antibacterial activity of Ethanol extract of *Azadirachta indica* showed maximum zone of inhibition (30mm) against *Salmonella typhii*, followed by *Escherichia coli* (24mm), *Pseudomonas aeruginosa* (20mm), *Staphylococcus aureus* (19mm)

The antibacterial activity of Acetone extract of *Azadirachta indica* showed maximum zone of inhibition (18mm) against Staphylococcus aureus, followed by Escherichia Coli (17mm), *Salmonella typhii* (16mm) and *Pseudomonas aeruginosa* (15mm). The antibacterial activity of Methanol extract of *Azadirachta indica* showed maximum zone of inhibition (20mm) against *Salmonella typhi*, followed by *Pseudomonas aeroginosa* (17mm), *Escherichia coli* (16mm) and *Staphylococcus aureus* (12mm).

Minimum Inhibition Concentration Test In Evaluation Of Anti-Microbial Efficacy

An antimicrobial skin-preparation according to the present invention was tested for anti microbial efficacy with the challenge gram positive and gram negative bacteria with resistance to various antibiotics. The minimum inhibition concentration test was also conducted to evaluate the long term effectiveness at killing microorganism.

- *Staphylococcus aureus* (ATTCC#6538) } Gram positive bacteria
- *Streptococcus pneumonia* (ATCC#35088)
- *Escherichia coli* (ATCC#11229) } Gram negative bacteria
- *Pseudomonas aeruginosa*(ATCC#27853)

Further the polyvinylpyrrolidone-iodine and polyvinylpyrrolidone-iodine *Azadirachta indica* by using anti-bacterial activity. Two strains including Gram negative E. coli, and Pseudomonas aeruginosa and Gram-positive Streptococcus pneumonia and Staphylococcus aureus were selected for antibacterial tests because they are usually associated with the medical-associated infections. The comparative Anti-bacterial property was investigated by calculating anti-bacterial ratios based on the numbers of bacteria colonies incubated with different dosages .The equivalent volumes of each of the bacterial cultures was added into each of the testing products (PVP-I and *Azadirachta indica*) and a series of 1:1 dilution of the mix made with sterile saline. The mixed materials were incubated for 24hrs to observe the microbial growth.

The electronic microscopic images was showed that the present invention (PVP-I and *Azadirachta indica*) produced similar antimicrobial inhibition activity to the control. The concentration of (PVP-I and *Azadirachta indica*) control that limit microbial growth to a specified amount over a 24hrs period.

Fig.4 Bacterial culture

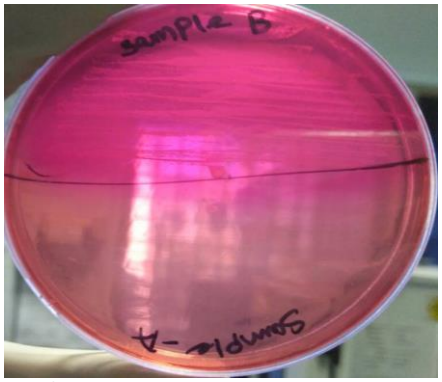

Fig.5 growth of microbial

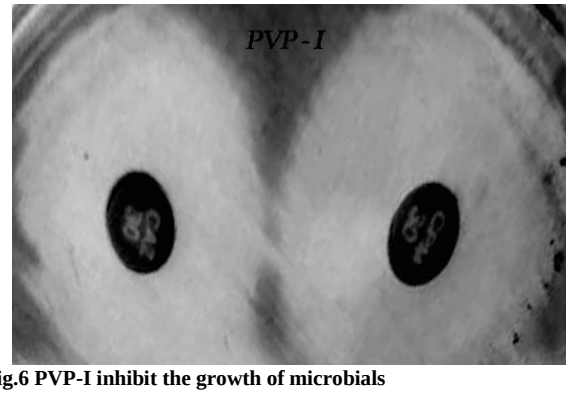

Fig.6 PVP-I inhibit the growth of microbials

Fig.7 Azadirachta inhibit the growth of microbials

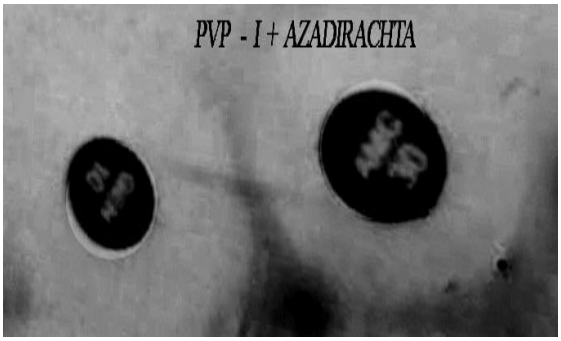

Fig.8 PVP-I and *Azadirachta indica* inhibit the growth of microbials

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