Antifungal activity of the seed coat extract of *Azadirachta indica*

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Purified fraction (ethyl acetate:chloroform; 3:1) of methanolic extract of *Azadirachta indica* seed coat showed antifungal activity against *Aspergillus niger* and *Curvularia lunata* with a minimum inhibitory concentration of 250 ppm. Among the four extracts (petroleum ether, chloroform, ethyl acetate and methanol) of leaves of *Azadirachta indica,* petroleum ether extract was found to be antifungal at 1000 ppm.

***AZADIRACHTA** indica*, commonly known as neem is a well known plant native to India. It has been extensively studied for its chemical constituents and their biological activity[1,2]. Recently there has been a dramatic increase in the fungal infections in human especially in immunocompromised and immuno-suppressed patients. Some plant extracts/products have been reported to be antifungal against different phytopathogenic and human disease causing fungi[3,4,5]. This has led us to work on plants especially *Azadirachta indica* for its antifungal activity. The present investigation deals with the evaluation of antifungal activity of different solvent extracts of seed coat and leaves of this plant.

Dried seed coat (coarse powder) was extracted with methanol at room temperature (30°) and the extract was obtained by evaporating the solvent under reduced pressure. The methanolic extract was subjected to silica gel column chromatography and eluted with different solvents such as petroleum ether, benzene, chloroform, ethyl acetate and methanol. The crude extracts were tested for antifungal activity using TLC bioassay[6]. Further ethyl acetate extract was chromatographed over silica gel column and eluted with mixtures of chloroform and ethyl acetate.

Dried leaves were extracted with methanol at room temperature (30°) and solvent evaporated under reduced pressure. The extract was taken in water and extracted successively with petroleum ether, chloroform, ethyl acetate and methanol. The extract obtained were subjected to antifungal evaluation using the method as above[6], applying the different extracts on TLC plates, over spraying the spore suspension of the test fungi and subsequently incubating at 25±2° for 3-4 days. Presence of growth inhibition zone around the test sample was indicate of antifungal activity of the test sample. Minimum inhibitory concentration (MIC) is defined as minimum concentration at which fungal growth inhibition zone was observed.

The ethyl acetate extract obtained from *Azadirachta indica* seed coat was found to be antifungal. Further fractionation of the ethyl acetate extract on silica gel column and elution with chloroform-ethyl acetate afforded different fractions. On evaluation of their antifungal activity, chloroform-ethyl acetate (3:1) fraction was found to be Antifungal with a minimum inhibitory concentration (MIC) of 250 ppm against both *Aspergillus niger* and *Curvularia lunata.*

The result obtained with leaf extract showed that petroleum ether extract had antifungal activity with a MIC of 1000 ppm, whereas, chloroform, ethyl acetate and methanol extracts were not found antifungal even at a concentration of 2000 ppm.

This is the first report on the antifungal activity of the seed coat extract of *Azadirachta indica*. The result obtained in this study indicate the presence of antifungal compound in different parts of *Azadirachta indica*. In the present study, the Antifungal activity of seed coat extract was more than

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that of leaf extract suggesting the possible use of seed coat extract for further evaluation and purification in search of active principle.

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Differential Pulse Polarographic Determination of 1,4-Benzodiazepine Psychotropic Drugs in Pharmaceutical Formulations and urine Samples

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A simple, rapid, and sensitive differential pulse polarographic method is developed for the determination of 1,4-benzodiazepine psychotropic drugs such as pinazepam, prazepam and temazepam in pharmaceutical formulations and urine samples, by the aid of universal buffers of pH 2.0 to 12.0. The developed procedure has been applied for the determination of these drugs in pharmaceutical formulations and urine samples as well as simultaneous determination in a single run.

SINCE the introduction of chlordiazepoxide hydrochloride in 1960, a large number of 1,4-benzodiazepine compounds have been investigated as tranquilizers, hypnotics, sedatives and antidepressants[1]. The 1,4-benzodiazepines are usually present in trace amounts following therapeutic administration because they undergo extensive biotransformation and tissue distribution. Pinazepam [7-chloro-1,3-dihydro-5-phenyl-1- (prop-2-ynyl)-2H-1, 4-benzodizepin-2-one], prazepam [7-chloro-1-cyclopropylmethyl)-1,3-dihydro-5-phenyl-2H-1, 4-benzodiazepin-2-one] and temazepam [7-chloro-1,3-dihydro-5-phenyl-1-methyl-2H-3-hydroxy-1,4-benzodiazepin-2-one] all have more pronounced psychotropic actions than other benzodiazepines, and they are also used in small doses. Therefore, a reliable analytical method is needed for the accurate determination of these drugs. Several methods have been described for the determination of benzodiazepines in biological fluids which are based on electron capture gas liquid chromatography[2], luminescence determination on thin layer chromatographic plates[3], high pressure liquid chromatography[4] and spectrophotometry[5]. The aim of the present work is to study the differential pulse polarographic behaviour of the title compounds and use it for their determination in pharmaceutical formulations and urine samples.

The details of the equipment used for the present investigation, theoretical, experimental procedures of these techniques were discussed elsewhere[6]. The polarographic behaviour of pinazepam, prazepam and temazepam was examined over the pH range 2.0 to 12.0. All these