

## Antimicrobial and antimolluscal activities and assessment of the active constituents of plant latices and their sera

[Ahmed A. M. Shoreit , Mady A. Ismail , Nadia H. Mohamed]

**Abstract**— The effect of three plant latices on some pathogenic bacteria, dermatophytes and phytopathogenic fungi, and two medically important species of molusca as well were studied. Trials for detection of the active compounds and/or enzymes in plant latex responsible for the antimicrobial activities were also carried out. The results revealed that the three plant latices have good antimicrobial activities on *Pseudomonas aeruginosa*, *Escherichia coli*, *Trichophyton rubrum* and *Chrysosporium tropicum*. Latex of *Ficus nitida* was the most effective against both bacterial and fungal species. Phytochemical analyses revealed the presence of several active compounds (glycosides, terpenoids and tannins) in the three plant latices, (saponins) in *Euphorbia pulcherrima* and *Ficus elastica*, (phenolic compounds and alkaloids) in *E. pulcherrima* and *F. nitida*, (flavonoids) in only *F. nitida* latex. The serum fraction of latex from both *E. pulcherrima* and *F. nitida* had protease, lipase, chitinase and cellulases ( $C_1$  and  $C_x$ ) enzymes. Three different bands were obtained from the *F. nitida* serum when fractionated by preparative gel electrophoresis, and these bands were tested separately and showed antifungal activity. Casein incorporated into separation gel was degraded and appeared as a clear zone around one of the three bands (the protease band, the first one) and this indicates the presence of protease enzyme in the serum of *F. nitida* latex. The presence of such active compounds or enzymes in the latices may explain the antimicrobial activity of these latices. Latices of *E. pulcherrima* and *F. nitida* have potent molluscicidal activities on both medically important species of *Biomphalaria alexandrina* and *Lymnaea truncatula*.

**Keywords**— latex, latex sera, phytochemical analysis, antimicrobial activity, mollusca, latex enzymes.

### Introduction

Latex is accumulated in over 2000 species of plants, and the latex-producing plant families are Euphorbiaceae, Asclepiadaceae, Moraceae, Asteraceae, Caricaceae, Cannabinaceae and Apocyanaceae<sup>[1]</sup>. The chemical composition of latex is very complex, it contains (wt/wt) natural rubber 25 to 35%, proteins 1 to 1.8% resins, fatty acids, sugars 1 to 2%, enzymes, alkaloids, terpenoids, tannins, lipids 0.4 to 1.1%, polar lipids 0.5 to 0.6%, minerals 0.4 to

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0.6%, inorganic components 0.4%, amino acids, amides and 50 to 70% water<sup>[2]</sup>.

Many biologically active compounds are present in several plant latices<sup>[3]</sup>. The biological activity have been traced back in many cases to the presence of certain types of diterpenes, triterpene derivatives, alkaloids, glucosinolates, tannins, diterpene ester, saponins, phenolics and flavonoids and many of them may contribute to antimicrobial properties<sup>[4,5]</sup>. Various latex from several plant species are used in folk medicine such as those from *Euphorbia* spp., *Ficus* spp., *Calotropis procera*, *Carica papaya*, *Hevea brasiliensis*, *Lactuca sativa*, *Asclepias curassavica*, exhibited antifungal<sup>[6,12]</sup>, antibacterial<sup>[13,14]</sup>, molluscicidal<sup>[15,16]</sup> activities. Latices are also reported to possess insecticidal, anti-inflammatory, antipyretic, analgesic, antimalarial, anti-parasitic, antiseptic and germicidal properties and as a filling material in the cavities of decayed teeth to check infection to stop the progress of cavities. Latex are also used as a traditional medicine against liver fluke, schistosomiasis in sheep, cattle, and even humans<sup>[15]</sup>, and for treatment of cancers, tumors, migraine, skin diseases, gonorrhea, swelling and warts remover and as antidiarrhoeal. A wide range of enzyme activity is found in latex fluids, such as proteases<sup>[14]</sup>, serine and cysteine proteinases<sup>[17,18]</sup>, and papain in the latex of *C. papaya*<sup>[19]</sup>. Ficin has been used variously to describe the crude dried latex from different species of the genus *Ficus*. The latex from species of *Ficus* had been reported to contain as many as 10 different sulfhydryl proteinases<sup>[20]</sup>. Plant latices also contain lipases<sup>[19,21]</sup>. Also, Sheldrake and Moir<sup>[20]</sup> found that *Hevea* Latex contain a highly active cellulase capable of hydrolysing carboxymethyl cellulose. High concentrations of antimicrobial substances are found in the latex such as chitinase/lysozymes<sup>[22]</sup>, which are capable of degrading the chitin component of fungal cell walls (chitin) and the peptidoglycan component of bacterial cell walls (lysozymes). Chitinases were also detected in *Carica papaya*, *Ficus microcarpa*<sup>[11]</sup>, and *Ficus carica* latices<sup>[23]</sup>. This work aimed to assess the antimicrobial (antibacterial, antifungal) and antimolluscal activities of the plant latices and their sera. Trials for determination of the active compounds and/or enzymes of plant latex responsible for the antimicrobial activities including phytochemical analysis of the plant latex and detection of protease, lipase, chitinase and cellulases ( $C_1$  and  $C_x$ ) enzymes activity of the fractionated latex serum were also studied.

### Materials and Methods

#### Latex plants used and their descriptions

*Euphorbia pulcherrima* Willd. ex Koltzsch (family Euphorbiaceae), *Ficus elastica* Roxb. ex Hornem and *Ficus*



*nitida* Thunb (family Moraceae) have been chosen for this study. Latices from the above plants grown in both Faculties of Science and Agriculture farms, University of Assiut were collected from plants (seasonally during autumn, winter 2006, spring and summer 2007) by tapping method [24].

#### **Fractionation of plant latex**

Fresh latex was centrifuged and separated as described in [25].

#### **Antimicrobial activity**

##### **Test organisms**

Seven clinical isolates of bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *B. subtilis*, *Micrococcus luteus*, *Staphylococcus albus* and *Serratia* sp.) and ten fungal isolates of dermatophytes (*Trichophyton rubrum* AUMC 1804, *Chrysosporium tropicum* AUMC 1807, *Microsporum canis* AUMC 2805, *Candida albicans* AUMC 3880, *Arthroderma grubyi* AUMC 2349, *A. racemosum* AUMC 2352) and phytopathogenic (*Fusarium solani* AUMC 222, *F. oxysporum* AUMC 210, *Colletotrichum gloeosporioides* AUMC 2779 and *Macrophomina phaseolina* AUMC 236) were used in this study. Bacterial and fungal isolates were kindly obtained from Microbiology lab, Faculty of Medicine, and Assiut University Mycological Centre (AUMC), respectively. Cultures of bacteria and fungi were sub-cultured on nutrient agar and Sabouraud dextrose agar slants respectively, and stored at 4°C until required. The agar well diffusion method [26] was used. The plates were incubated at 37°C for 24 hours for the bacteria, and at 25°C for 6 days for fungi. The zones of inhibition were measured in mm. The lowest concentration of the plant latex that inhibited the growth of the test organisms was recorded as the minimum inhibitory concentration (MIC) [14].

##### **Antimolluscal activity**

The experiment was performed according to the methodology standardized by WHO [27]. Two species of snails (*Biomphalaria alexandrina* and *Lymnaea truncatula*) were tested. Crude latex of *E. pulcherrima* and *F. nitida*, and latex serum were prepared as described above. The fractions were used in four different forms: vital (2 forms): (1) the serum without any treatment, (2) pellet resulted from precipitation of vital serum with acetone; and the denaturated boiled (2 forms): (3) the serum after boiling for 20 min and (4) pellet resulted from precipitation of vital serum with acetone then boiling for 20 min. These forms were used for studying their antifungal activity on *T. rubrum* and *C. tropicum* using agar well diffusion method.

#### **Methods used for detection of different enzymes in latex sera**

Latex sera of the three different plants *E. pulcherrima*, *F. elastica* and *F. nitida* were screened for their abilities to produce protease (caseinase), cellulases ( $C_1$  and  $C_x$ ), lipase and chitinase enzymes.

**Protease activity:** Latex proteolytic activity was tested using casein hydrolysis medium [28].

**Lipase activity:** Latex lipolytic activity was measured using the medium of Ullman & Blasins [29] with slight modification.

**Cellulases activity: A) Test for exo-1, 4- $\beta$ -glucanase ( $C_1$ ) production:** The medium described by Eggs & Pugh [30] was

used to induce the production of cellulose components especially the exo-1, 4- $\beta$ -glucanase ( $C_1$ ). The cellulose agar plates were inoculated as in case of protease production.

**B) Test for endo-1, 4- $\beta$ -glucanase ( $C_x$ ) production:** Utilization of soluble cellulose by the cellulase enzyme that may be present in latex serum was detected on a modified medium of Prasad & Verma [31].

**Chitinase activity:** Sugar-free Czapek agar medium of Lynch [32] amended with 2 g/l colloidal chitin prepared as described by Hsu & Lockwood [33] was used for testing the ability of latex serum to digest chitin and produce of chitinase enzyme.

#### **Gel electrophoresis for separation of different serum fractions of *Ficus nitida* latex**

##### **Native preparative gel electrophoresis of latex serum of *F. nitida* and study of its antifungal and different enzyme activities**

The protein latex serum was analyzed using preparative native gel electrophoresis without SDS (12% native-PAGE) following the method of Laemmli [34]. Activity staining was done according to the protocol followed by Felicioli [35].

##### **Detection of protease enzyme on native gel electrophoresis of latex serum of *F. nitida***

Casin-PAGE for protease activity was performed by including casin (0.5% w/v final concentration) to 12% native-polyacrylamide gel as described by Devaraj [36].

##### **Phytochemical analyses of plant latex**

The latex samples of *E. pulcherrima*, *F. elastica* and *F. nitida* were analyzed for their phytochemical composition by qualitative methods of Mahajan & Badgujar [4].

#### **Results and Discussion**

##### **Antimicrobial activity of plant latices collected during different seasons of 2006/2007**

###### **Antibacterial activity**

The latices of the three plants (*Euphorbia pulcherrima*, *Ficus elastica* and *Ficus nitida*) collected during the four seasons were tested against strains of *Bacillus cereus*, *B. subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus albus* and *Serratia* sp. exhibited no antibacterial activity upto 700  $\mu$ l concentrations against all except *E. coli* and *P. aeruginosa* strains. It was also noted that latices of the three plant species collected during spring and summer of the year 2007 have no antibacterial activity at all concentrations used (3-700  $\mu$ l latex / 0.5 or 1 cm diam well) against *P. aeruginosa* and *E. coli*. However, the latices collected during autumn 2006 and winter 2006/2007 have antibacterial activity at least on one of the two organisms. The minimum inhibitory concentration (MIC) of *E. pulcherrima* latex was 6.25  $\mu$ l during autumn and 3  $\mu$ l during winter for *Pseudomonas aeruginosa* while 25  $\mu$ l during autumn and 12.5  $\mu$ l during winter for *Escherichia coli* (Table 1). With respect to *F. elastica* latex, the MIC for *P. aeruginosa* was 50  $\mu$ l of latex collected during autumn and 25  $\mu$ l of latex collected during winter, while no effect was detected on *E. coli* by all concentrations used (upto 700  $\mu$ l). Regarding the MIC of *F. nitida* latex on *Pseudomonas aeruginosa*, it was 3  $\mu$ l of latex collected during autumn while it was 2  $\mu$ l during winter. On the other hand, for *E. coli* it was 6.25  $\mu$ l during autumn while 3  $\mu$ l c

current results it could conclude that, the three plant latices collected during winter were more effective than those collected during autumn. Also, latex of *F. nitida* was the most effective against both bacterial species. In this respect, Ajayi<sup>[13]</sup> reported antibacterial activity of *Ficus asperifolia* latex on *Bacillus polymyxa* and *Escherichia coli*. Also, Kareem<sup>[14]</sup> showed that the aqueous, ethanol and chloroform extracts of *Calotropis procera* latex exhibited bacteriocidal effects on the pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, *S. albus*, *Streptococcus pyogenes* and *S. pneumoniae*. Moreover, Osato et al.<sup>[37]</sup> found the latex of *Carica papaya* to be bacteriostatic to *Bacillus subtilis*, *E. coli*, *Enterobacter cloacae*, *Salmonella typhi*, *Staphylococcus aureus* and *Proteus vulgaris*. Also Nair & Chanda<sup>[38]</sup> screened the water and ethanol extracts of the bark collected from *Ficus religiosa*, *F. racemosa*, *F. benghalensis* and *F. tiselata* for potential antibacterial activity against the medically important bacterial strains, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Alcaligenes faecalis* and *Salmonella typhimurium* and found that the maximum activity was shown by those of *F. racemosa*, *F. benghalensis* and *F. tiselata* against the bacterial strains tested. The current results that the latices collected during spring and summer seasons have no antibacterial activities are similar to those obtained when using the latex of *Alstonia boonei*<sup>[39]</sup> or methanolic leaf extract of *Alstonia boonei*<sup>[40]</sup>. These authors observed that the microbial colonies were larger than the colonies in control plates in all bacteria tested and this means with no doubt that the latex must have served as enrichment medium to the tested bacteria.

#### Antifungal activity

Latices of the three plants collected during the four seasons and tested against the 6 human fungal pathogens (*Microsporium canis*, *Candida albicans*, *Arthroderma grubyi*, *Arthroderma racemosum*, *Chrysosporium tropicum*, *Trichophyton rubrum*) and the 4 phytopathogenic fungal species (*Fusarium solani*, *F. oxysporum*, *Colletotrichum gloeosporioides*, and *Macrophomina phaseolina*) exhibited no antifungal activity (upto 700 µl concentrations) against these fungal strains except *T. rubrum* and *C. tropicum*. The MIC of *E. pulcherrima* latex was 1µl during autumn and winter for *T. rubrum* while 1µl during autumn and 0.5 µl during winter for *C. tropicum*. On the other hand, MIC for both *T. rubrum* and *C. tropicum* was 2 µl during spring and 3 µl during summer. Similar MICs of *F. nitida* latex on *T. rubrum* were recorded during autumn and winter (1µl) while it was 2 µl during spring and summer. On the other hand for *C. tropicum* it was 1 µl during both spring and summer while 0.5 µl during both autumn and winter seasons (Table 1). With respect to *F. elastica* latex, the MIC on both *T. rubrum* and *C. tropicum* was 6.25 µl during autumn and winter. Unexpectedly it was noted that *F. elastica* latex has no effect on both fungal species during spring and summer at all concentrations used (3-700 µl latex), the same case was reported for bacterial species. In this respect, latex of *Calotropis procera* had antifungal effects on *Aspergillus niger*, *A. flavus*, *Microsporium boulandii*, *Candida albicans*<sup>[14]</sup>, and latex from *Hevea brasiliensis* exhibited

antifungal activities against seven *Candida* species, *Cryptococcus neoformans*, *Trichosporon cutaneum* and *Saccharomyces cerevisiae* and the strong antifungal effect was obtained with *T. cutaneum* and *C. neoformans*<sup>[10]</sup>. Giordani et al.<sup>[41]</sup> found a synergistic effect between ketoconazole and *Euphorbia characias* latex against *Candida albicans*, and the antifungal activity of ketoconazole was enhanced by the latex. Giordani<sup>[8,9]</sup> explained the fungistatic effect of *Carica papaya* latex against *C. albicans* as a result of cell wall degradation due to a lack of polysaccharide constituents in the outermost layers of the fungal cell wall and release of cell debris into the culture medium. Also, Mavlonov<sup>[23]</sup> isolated and purified the antifungal protein from latex of *Ficus carica* and found that it exhibited antifungal activity against the phytopathogenic strains *Verticillium dahliae* and *Fusarium oxysporum*. Pereira et al.<sup>[7]</sup> presented also that latex from *Manihot glaziovii* had an inhibitory effect on the phytopathogenic fungi *Colletotrichum gloeosporioides*, *Fusarium solani* and *Macrophomina phaseolina*. These results suggest the presence of substances, some of them of protein nature, like enzymes and enzyme inhibitors which strongly interfere with the development of these fungi. Kumpoun et al.<sup>[12]</sup> found that latex of 'Mahajanaka' mango contained large amount of water insoluble fraction which contained antifungal compounds against *Colletotrichum gloeosporioides*, the cause of anthracnose of mango fruit.

#### Effect of crude latices and their fractions on *T. rubrum* and *C. tropicum*

Latices (crude and their fractions) collected during winter season (since they were more active than those collected during the other seasons) were used for further antifungal studies. Fresh latex, centrifuged at 17,000 rpm for 20 min at 4 °C, was separated into three layers: top layer containing natural rubber, middle fraction of serum, and bottom fraction called loutid (fig 1). Rubber fractions (top layers) of the three different plant latices showed no activity on both fungal species tested while bottom fractions (loutid) has little effect on these species. Crude latices and their serum fractions of the three plants possessed higher antifungal activity (than that of loutid) against both fungal species, however, the activity was slightly higher on *C. tropicum* than on *T. rubrum* in all cases. At the same time serum fraction was more active than the crude latex of the same plant latex on both fungi (Table 2). In this respect, Taira et al.<sup>[11]</sup> made fractionation of latex from *Ficus microcarpa* into a top layer of polymerized rubber, serum and a large bottom fraction and found that chitinase enzyme was detected in the serum fraction. The current results that serum fraction and crude latex had antifungal activity may be explained as due to the presence of chitinase enzyme.

#### Antimolluscal activity of three plant latices

*Biomphalaria alexandrina* is medically important because the snails can carry a parasite which represents a serious disease risk to humans; the snails serve as an intermediate host (vector) for the human parasitic blood fluke, *Schistosoma mansoni*. The fluke, which is found primarily in tropical areas, infects mammals (including humans) via contact with



water that contains schistosome larvae (cercariae) which have previously been released from the *Biomphalaria alexandrina* snail. Infection occurs via penetration of cercariae through the skin. In humans this fluke causes the debilitating disease schistosomiasis [42]. *Lymnaea truncatula* is the principal intermediate host of *Fasciola hepatica* due to fasciolosis [43]. The heavy uses of synthetic pesticides and synthetic pyrethroids affect the aquatic environment due to some properties such as high toxicity to target and non-target organisms and long-term persistence in the environment [44].

Latices of the three plant species collected during winter season were selected to study their activity on these two medically important mollusca species. A hundred percent mortality was achieved for *Biomphalaria alexandrina* after 48 hours of treatment by *E. pulcherrima* or *F. nitida* latex. On the other hand, after 48 hours of treatment of *Lymnaea truncatula*, a 100% mortality was achieved by *F. nitida* latex while only 90% by *E. pulcherrima* latex. On contrary, *F. elastica* latex has no effect on *B. alexandrina* snail even after 6 days of treatment, while on *L. truncatula*, 90% mortality was achieved after the same period of treatment (Table 3).

#### **Effect of vital, acetone treated and boiled serum of *E. pulcherrima* and *F. nitida* latices on *T. rubrum* and *C. tropicum***

What are the active compound(s) in latex serum fraction of *E. pulcherrima* and *F. nitida*?. To answer this question, trials for detection of the active compound and/or enzymes in plant latex responsible for the antimicrobial activity were carried out. Since serum fractions were more active against both fungi than crude latices of both *E. pulcherrima* and *F. nitida* and than those of *F. elastica*, These fractions were used for further study to assess the antimicrobial active compounds in these fractions. These fractions were used in four different forms: vital (2 forms): (1) the serum without any treatment, (2) pellet resulted from precipitation of vital serum with acetone; and the denaturated boiled forms (2 forms): (3) the serum after boiling for 20 min and (4) pellet resulted from precipitation of vital serum with acetone then boiling for 20 min. The vital forms (the not-treated and acetone-treated serum fractions) of both *E. pulcherrima* and *F. nitida* latices showed antifungal effects on both *T. rubrum* and *C. tropicum*, however the effect of both forms of the serum fraction was higher on *C. tropicum* than on *T. rubrum*. At the same time, buffered pellet of the acetone-treated serum fractions from both latex plants were more active on both fungi tested. Also the vital forms of the serum fraction of *Ficus nitida* were more effective on both fungal species than those of *E. pulcherrima*. On the other hand, the denaturated forms (the non- treated and acetone-treated boiled serum fractions) of both *E. pulcherrima* and *F. nitida* had no effect on both fungal species tested. The current results indicate that the antifungal properties of the vital forms of latex serum may be due to lytic enzymes in these fractions.

#### **Activity of enzymes produced by plant latex sera collected in winter**

For the reason that the crude latices collected during winter season 2006 – 2007 possessed the lowest values of minimal inhibitory concentration on both fungi and bacteria, i.e. the

higher (or at least the same) activities than those collected during the other seasons, the serum fraction of latices collected from the three different latex plants during winter season were screened on media containing different substrates for enzymes to assess the enzymes that may be found and may be responsible for the antifungal properties of these fractions. Results presented in table (5) revealed that the serum fraction of latex from both *E. pulcherrima* and *F. nitida* had protease, lipase, chitinase and cellulases ( $C_1$  and  $C_x$ ) enzymes. The activity of all enzymes detected in the serum fraction of *E. pulcherrima* latex was higher than that of *F. nitida*. On the other hand the serum fraction of *F. elastica* latex had only protease enzyme (out of the five enzymes screened) with a lowest amount of activity than that of *F. nitida* and *E. pulcherrima* (table 4). In agreement with the current results, lipolytic activities were detected for crude latex from five *Euphorbia* species on various triglycerides and for *Euphorbia characias* [22], with the highest activity being detected in the latices collected during the month of June. On the other hand, cellulase enzymes were detected in latex of *Hevea brasiliensis*, in the latex of species with articulated laticifers but not in the latex of species with nonarticulated laticifers [20,45]. Also, in agreement with the current finding that chitinase enzyme was detected in *E. pulcherrima* and *F. nitida* latex sera, Taiara et al. [11] found the chitinase enzyme in the serum fraction but of *Ficus microcarpa*.

#### **Native polyacrylamide gel electrophoresis and separation of protein produced by latex serum of *Ficus nitida* Antifungal and enzyme activities of serum fractions**

Three different bands were obtained from the serum of *Ficus nitida* latex when fractionated by preparative gel electrophoresis (Fig. 2). These bands were separately tested against *T. rubrum* and *C. tropicum* and found effective, however band 1 and band 3 were slightly more effective than band 2. Also these bands were tested for their enzyme activity on agar plates and all of them possessed, but with different capabilities, lipolytic, cellulolytic and chitinolytic activities. The three bands showed these activities in the following order cellulase, lipase and chitinase. However, the proteolytic activity was proved by only the first band with RF 0.19 (Table 5 & Fig. 2). The glycosidase activities (as  $\alpha$ -mannosidase and N- acetyl- $\beta$ -D-glucosaminidase) associated with the crude latex of *Hevea brasiliensis* [10], and *Carica papaya* [46] support the hypothesis of an enzymic hydrolysis of key structural polymers such as chitin in the fungal cell wall. The role of other enzymes such as protease and lysozyme located in the *Hevea brasiliensis* latex can partially explain its antifungal effect [47]. The chitin-binding domain of basic class-I chitinase found in the latex of *Ficus microcarpa* and other latex fluids has been shown to exhibit antifungal activity, since it binds to the chitin in cell walls of many fungi. These chitinases inhibited hyphal extension of several fungal pathogens, *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani*, as well as *Trichoderma viride* [11]. Chitin-binding antifungal protein was also isolated and purified from *Ficus carica* latex and found to exhibit antifungal activity against the phytopathogenic cotton wilt fungi *Verticillium dahlia* and

*Fusarium oxysporum* [23]. Parijs et al. [6] isolated also several chitin-binding proteins from *Hevea brasiliensis*. One of these proteins is hevein, showed strong activity against several fungi *in vivo* and/or *in vitro*. The possible involvement of this protein in the defense against invasion by potentially pathogenic fungi was keyed out [48].

#### Protease activity of latex serum of *F. nitida* as detected by native gel electrophoresis

Casein incorporated into separation gel was degraded and appeared as a clear zone around one of the three bands (the 1st, the protease band with RF 0.19) after staining the gel by Commassie brilliant blue. This indicates that protease (caseinase) enzyme is found in the first band of the serum of *F. nitida* latex (Fig. 2).

It is well known that latices commonly contain several enzymatic and inhibitory activities of potential action against insects and pathogens [7]. Yadav et al. [18] found also that the latex of *Euphorbia milii* exhibited high caseinolytic activity, indicating the presence of protease (s). Moreover, Devaraj et al. [36] purified and characterized of a protease from the latex of *Ficus racemosa* using Gelatin-PAGE. Proteases in the latex of several *Euphorbia* species (including *E. pulcherrima*) as serine protease [18], in the latex of *Ficus glabrata*, five sulfhydryl endopeptidases, one of them called ficin [20], in the latex of medicinal plant *Calotropis procera* [17], in the latex of *Funastrum clausum* (Asclepiadaceae) cysteine endopeptidase, named funastrain [49], in the latex of *Carica papaya* (e.g. papain, chymopapain and endopeptidase), in the latex of *Vasconcellea* spp. (The cystein proteases) have been reported [19].

#### Phytochemical analysis of plant latices

Latices of *E. pulcherrima*, *F. elastica* and *F. nitida* consist of a suspension of particles with pH 6.3, 5.4 and 5.9, respectively. Silva et al. [50] found that pH of *Himatanthus sucuuba* latex 4.54. Protein, glycosides, terpenoids and tannins have been found in the three latices, while alkaloids and phenolic compounds were found only in case of *E. pulcherrima* and *F. nitida* latex. On the other hand, flavonoids were found only in *F. nitida* latex, while saponins were found in *E. pulcherrima* and *F. elastica* latices (Table 7). Our results are in agreement with those of Mahajan & Badgujar [4] who carried out phytochemical analyses on latex obtained from twenty one plant species (species of *Ficus* and *Euphorbia* were included) for the presence of possible secondary metabolites and found phenolic compounds in all latex samples except in *Ipomoea carnea*. Of these, 76% of latex plants contain alkaloids. Terpenoids were found in the latex of *Carica papaya* and *Manilkara zapota*. Cynogenic glycosides and tannins were detected in 11 and 10 of latex plant species, respectively. The whole plant latex obtained from *Euphorbia hirta* showed presence of alkaloids, phenolics, tannins and flavonoids and absence of terpenoids, cynogenic glycosides and saponins. In the current study, terpenoids are found in the latex of *E. pulcherrima*. In this respect, many of the *Euphorbia* species have been the object of chemical and pharmacological investigations because of the irritant and carcinogenic properties of their latex [51]. These biological

properties have been traced back in many cases to the presence of certain types of diterpenes [4]. Abdel-Monem et al. [52] isolated also three triterpenoids from the chloroform fraction of the alcoholic extract of *Euphorbia schimper*. Several diterpene and triterpene were also isolated from *Euphorbia lacteal* and many of them were found to possess antitumor activity [53]. Bigoniya & Rana [5] isolated several triterpenes from latex, bark, and root of *Euphorbia neriifolia*. Giordani et al. [41] showed that the terpenic secretion particles of latex from *Asclepias curassavica* to be implicated in the antifungal action against *Candida albicans*. In agreement with the current results on *E. pulcherrima*, Falodun et al. [54] found saponins and tannis in *Euphorbia heterophylla* leaf. However, they found also flavonoids which are absent in *E. pulcherrima* latex in our work. We think that the phytochemical analysis of the plant latices, presented in the current study is useful to researchers and in industry regarding pharmaceuticals.

Figure 1. Fractionations of latex of *Euphorbia pulcherrima* (A), *Ficus elastica* (B), *Ficus nitida* (C) and natural rubber of *F. elastica* after preparation (D).

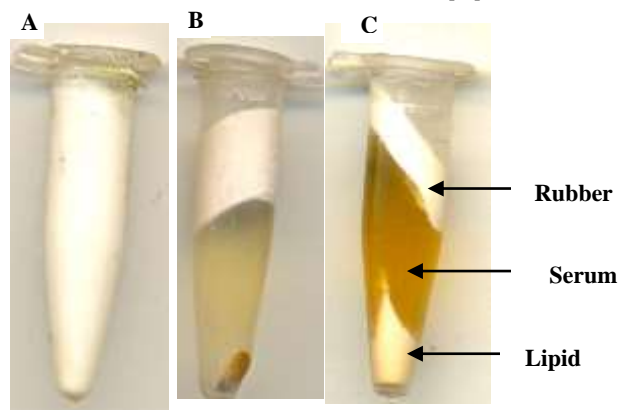


Figure 2. Preparative and Native page gel electrophoresis of *F. nitida* latex serum

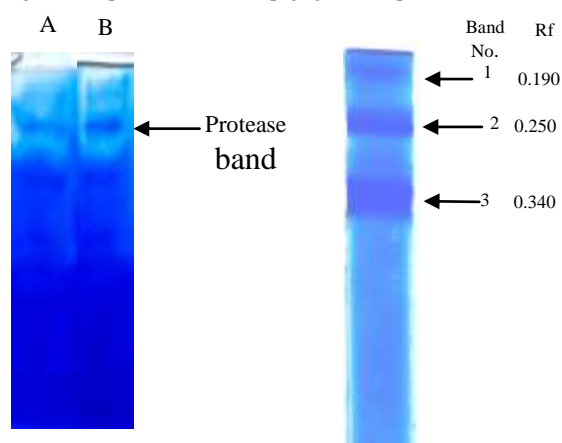


Table 1. Summarized data for MICs of the three plant laticies at concentration of 50 µl in winter on *Trichophyton rubrum* and *Chrysosporium tropicum*

Seasons	Autumn 2006			Winter 2006/2007			Spring 2007			Summer 2007		
	EP	FE	FN	EP	FE	FN	EP	FE	FN	EP	FE	FN
<i>Ps.aerugi nosa</i>	6.25	50	3	3	25	2	NA	NA	NA	NA	NA	NA
<i>E. coli</i>	25	NA	6.2 5	12.5	NA	3	NA	NA	NA	NA	NA	NA
<i>T. rubrum</i>	1	6.2 5	1	1	6.2 5	1	2	NA	2	3	NA	2
<i>C. tropicum</i>	1	6.2 5	0.5	0.5	6.2 5	0 5	2	NA	1	3	NA	1

- FE= *Ficus elastica*, EP= *Euphorbia pulcherrima* and FN= *Ficus nitida*
- NA=not active up to the highest concentration used (700 µl / 1 cm diameter well).

Table 2. Effect of crude latices and their serum of *E. pulcherrima*, *F. elastica* and *F. nitida*.

Test organisms (Fungi)	<i>Trichophyton rubrum</i>		<i>Chrysosporium tropicum</i>	
	Crude latex	Serum	Crude latex	Serum
<i>E. pulcherrima</i>	19.6±0.81	22.3±0.81	21.5±1.04	25.5±1.05
<i>F. elastica</i>	9.5±0	10±0.8	10.3±0	11.6±1
<i>F. nitida</i>	20±1.24	30±1.26	25.8±0.75	32±0

- Figures are mean diameters of inhibition zones (in mm) ± standard deviation
- Table 3. Antimolluscal activity of plant latices against two mollusca species at concentration 2 ml/500 ml distilled water.

Latex source	Duration of treatment	Mortality of			
		<i>Biomphalaria alexandrina</i>		<i>Lymnaea truncatula</i>	
		Dead/Exposed	%	Dead/Exposed	%
<i>E. pulcherrima</i>	24 hr	7 / 10	70	8 / 10	80
	48 hr	10 / 10	100	9 / 10	90
<i>F. elastica</i>	6 days	0 / 10	0	9 / 10	90
<i>F. nitida</i>	24 hr	9 / 10	90	7 / 10	70
	48 hr	10 / 10	100	10/10	100

- Treated snails were rinsed in the diluted latex (2 ml latex/500 ml distilled water).
- Control snails were rinsed in latex- free distilled water.

Table 4. Activity of enzymes produced by latex sera

Enzyme	<i>E. pulcherrima</i>	<i>F. elastica</i>	<i>F. nitida</i>
Protease	10.8±1	5.6±0.5	9.6±0.5
Lipase	18±0.5	0	17±1
C1	14.1±0.9	0	7±0.6
Cx	9.1±0.057	0	7.1±0
Chitinase	6±0.4	0	6±0.6

- Production of protease, cellulase and chitinase were recorded as mean diameters of clear zones (mm) ± standard deviation (SD) (around the serum fraction added, 50 µl /5mm diameter well).

- Lipase production was detected as the depth of the visible precipitate of the test media (mm) formed due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme found in the serum fraction of the latex.

Table 5. Antifungal and enzyme activities of fractionated serum of *F. nitida* latex by preparative gel electrophoresis.

Protein bands/ Test fungi and enzymes	Band 1	Band 2	Band 3
<i>Trichophyton rubrum</i>	7±0.9	6±0	7.3±0.5
<i>Chrysosporium tropicum</i>	7.5±0.5	6.6±0.5	7.5±0.5
Protease	15.1±0.7	0.0	0.0
Lipase	8.3±0.8	8±0	8.3±0
Cellulase	12.3±0.5	9.6±1.1	12±1
Chitinase	6.5±0.5	6±1	6.5±0.5

- Figures are mean diameters of inhibition zones (in mm) ± standard deviation

Table 6. Phytochemical analysis of the plant latices

plant latex	<i>E. pulcherrima</i>	<i>F. elastica</i>	<i>F. nitida</i>
pH	6.3	5.4	5.9
Protein	+	+	+
Alkaloids	+	-	+
Glycosides	+	+	+
Phenolics	+	-	+
Flavonoids	-	-	+
Terpenoids	+	+	+
Tannins	+	+	+
Saponins	+	+	-

## References

- [1] T.M. Lewinsohn, "The geographical distribution of plant latex" *Hemoecology*, vol. 2, 1991, pp.64-68.
- [2] A. Subramaniam, "The chemistry of natural rubber latex" *Immunol Allergy Clin N Am*, vol. 15, 1995, pp. 1-20.
- [3] S. Öksüz, A. Ulubelen, A. Barla, "Terpenoids and aromatic compounds from *Euphorbia heteradena*" *Turk J Chemist*, vol. 26, 2002, pp. 457-463.
- [4] R.T. Mahajan, S.B. Badgular, "Phytochemical investigation of some laticiferous plants belonging to khandesh region of maharashtra" *Ethnobot Leaflets*, vol. 12, 2008, pp. 1145-1152.
- [5] P. Bigoniya and A.C. Rana, "A Comprehensive phytopharmacological review of *Euphorbia nerifolia* Linn." *Pharmacognosy Reviews*, vol. 2 (4), 2008, pp. 57-66.
- [6] J.V. Parijs, F.W. Broekaert, I.J. Goldstein, W.J. Peumans, "Hevein: an antifungal protein from rubber-tree (*Hevea brasiliensis*) latex" *Planta*, vol. 183, 1991, pp. 258-264.
- [7] I.S. Pereira, V.G. Gomes, K.V.S. Fernandes, M.P. Sales, J. Xavier-Filho, "Insecticidal and antifungal proteins of the latex from *Manihot glaziovii* (Muell)" *Revt Bras Bot*, vol. 22, 1999, pp. 1-8.
- [8] R. Giordani, M.L. Cardenas, J. Moulin-traffort, P. Regli, "Fungicidal activity of latex sap from *Carica papaya* and antifungal effect of D (+) glucosamine on *Candida albicans* growth" *Mycoses*, vol. 39, 1996, pp. 103-110.
- [9] R. Giordani, C. Gachon, J. Buc, P. Regli, J.L. Jacob, "Antifungal action of *Hevea brasiliensis* latex. Its effect in combination with fluconazole on *Candida albicans* growth, *Mycoses*" vol. 42, 1999, pp. 465-474.
- [10] R. Giordani, Regli P., Buc J., "Antifungal effect of *Hevea brasiliensis* latex with various fungi: Its synergistic action with amphotericin B against *Candida albicans*" *Mycoses*, vol.45, 2002, pp. 476-481.
- [11] T. Taira, A. Ohdomari, N. Nakama, M. Shimoji, M. Ishihara, "Characterization and antifungal activity of *Hevea brasiliensis* (*Ficus microcarpa*) latex chitinases: both the chi



- activities of class I chitinase are reinforced with increasing ionic strength, Bioscience" Biotechnol Biochem, vol.69 (4), 2005, pp. 811-818.
- [12] W. Kumpoun, J. Uthaibutre, D. Boonyakiat, "Antifungal compound content in mango latex at different maturity stages" ISHS Acta Horticulturae, 2007, pp. 804.
- [13] A.O. Ajayi, "Antimicrobial nature and use of some medicinal plants in Nigeria" Afr J Biotechnol, vol.7 (5), 2008, pp. 595-599.
- [14] S.O. Kareem, I. Akpan, O.P. Ojo, "Antimicrobial activities of *Calotropis procera* on selected pathogenic microorganisms" Afr J Biomed Res, vol.11, 2008, pp. 105-110.
- [15] V.T. Schall, M.C. Vasconcellos, R. S. Rocha, C.P. Souza, N. M. Mendes, "The control of the scistosometransmitting snails *Biomphalaria glabrata* by the plant molluscicide *Euphorbia splendense* var. *hislopii* (syn *E. milii* Des. Moul): a longitudinal field study in an endemic area in Brazil" Acta Tropica, vol. 79, 2001, pp. 165-170.
- [16] S.C. Yadav, M.V. Jagannadham, "Physiological changes and molluscicidal effects of crude latex and Milin on *Biomphalaria glabrata*" Chemosph, vol. 71, 2008, pp. 1295-1300.
- [17] V.K. Dubey, M.V. Jagannadham, Procerain, "a stable cysteine protease from the latex of *Calotropis procera*" Phytochem, vol.62 (7), 2003, pp. 1057-1071.
- [18] S.C. Yadav, M. Pande, M.V. Jagannadham, "Highly stable glycosylated serine protease from the medicinal plant *Euphorbia milii*" Phytochem, vol. 67, 2006, pp. 1414-1426.
- [19] M.E.L. Azarkan, A. Moussaoui, D.V. Wuytswinkel, G. Dehon, Y. Looze, Fractionation and Purification of the enzymes stored in the latex of *Carica papaya*" J Chromatography B, vol.790, 2003, 229-238.
- [20] R. Sheldrake, G.F.J. Moir, "A Cellulase in *Hevea* Latex" Physiol Plant, 1970, 23 (2), 267-277.
- [21] C. Palocci, S. Soro, E. Cernia, F. Fiorillo, C. Belsito, B. Monacelli, M. G. Delle, G. Pasqua, "Lipolytic isoenzymes from *Euphorbia* latex" Plant Sci, vol. 165, 2005, 577-582.
- [22] T. Subroto, S. Sufiati, J.J. Beintema, *Papaya* (*Carica papaya*) Lysozyme is a member of the family 19 (Basic, Class II) chitinases, J Molecular Evolution, vol.49 (6), 1996, pp. 819-821.
- [23] G.T. Mavlonov, K.A. Ubaidullaeva, M.I. Rakhmanov, "Chitin-binding antifungal protein from *Ficus carica* latex" Chemis Natural Compounds, vol.44 (2), 2008, pp. 171-173.
- [24] D. Wititsuwannakul, R. Wititsuwannakul, Biochemistry of natural rubber and structure of latex, In: Koyama T., Steinbuchel, A. (Eds.), Biopolymers, Polyisoprenoids, Wiley-VCH, Germany, vol.2, 2001, pp. 151- 201.
- [25] H. Kang, M.Y. Kang, K.H. Han, "Identification of natural rubber and characterization of rubber biosynthetic activity in fig tree" Plant Physiol, vol.123, 2000, pp. 1133-1142.
- [26] C.A. Omenka, J.O. Osuoha, "Antimicrobial potency of Grapefruit seed extract on five selected pathogens" Nigerian J Microbiol, vol.14 (2), 2000, pp. 39-42.
- [27] WHO (WORLD HEALTH ORGANIZATION), The control of Schistosomiasis. World Health Organ Tech Rep Ser 830 1994,
- [28] R.R.M. Paterson, P.D. Bridge, "Biochemical techniques for filamentous fungi" Int. Mycol. Instit. CAB international, Surrey, UK. 1994, (P.21).
- [29] V. Ulman, G. Blasins, "A simple medium for the detection of different lipolytic activity of microorganisms" Zentrabl Bakteriol Hyg Abt A vol. 229, 1974, 264-267.
- [30] H.O.W. Eggins, G.J.F. Pugh, "Isolation of cellulose decomposing fungi from soil" Nature, vol. 193, 1962, pp. 94-55.
- [31] J.S. Prasad, R.A.B. Verma, "Investigation on the diseases of papa. III- studies on pectolytic and cellulolytic enzymes production in vivo and in vitro by six pathogens" Physiology of Parasitism, 1979, Today and Tomorrows Printer's and publishers, India.
- [32] J.M. Lynch, J.H. Slater, J.A. Bennett, S.H.T. Harper, "Cellulase activities of some aerobic microorganisms isolated from soil" J Gen Microbiol, vol. 127, 1981, pp. 231-236.
- [33] S.C. Hsu, J.L. Lockwood, "Powdered chitin agar as a selective medium for enumeration of Actinomycetes in water and soil" Appl Microbiol, 1975, pp. 422-426.
- [34] V.K. Laemmli, "Cleavage of structural proteins during assembly of the head of bacteriophage T4" Nature, vol. 277, 1970, pp. 680-685.
- [35] R. Felicioli, B. Garzelli, L. Vaccari, D. Melfi, E. Balestreri, "Activity staining of protein inhibitors of proteases on gelatin containing polyacrylamide gel electrophoresis" Anal Biochem, vol. 244, 1997, pp. 176-179.
- [36] K.B. Devaraj, L. R. Gowda, Prakash V., "An unusual thermostable aspartic protease from the latex of *Ficus racemosa* L." Phytochem, vol. 69 (3), 2008, pp. 647-655.
- [37] J.A. Osato, L.A. Santiago, G.M. Remo, M.S. Cuadra, A. Mori, "Antimicrobial and antioxidant activities of unripe papaya" Life Sci, vol. 53, 1993, pp. 1383-1389.
- [38] R.Nair, S.V. Chanda, "Antibacterial activities of some medicinal plants of the western region of India" Turk J Biol, vol. 31, 2007, pp. 231-236.
- [39] P.O. Adomi, "Screening of the leaves of three Nigerian medicinal plants for antibacterial activity" Afr J Biotechnol, vol. 7 (15), 2008, pp. 2540-2542.
- [40] K. Anani, J. Hudson, C. Desouza, K. Akpagana, G.H.N. Towers, J.T. Arnason, M. Gbeassor, "Investigation of medicinal plants of Togo for antiviral and antimicrobial activities" Pharmaceutical Biol, vol. 38, 2000, pp. 40-45.
- [41] R. Giordani, J. Trebaux, M. Masi, P. Regli, "Enhanced antifungal activity of ketoconazole by *Euphorbia characias* latex against *Candida albicans*" J Ethnopharmacol, vol. 78 (1), 2001, pp. 1-5.
- [42] A.A. Hussein, S.M. Bin-Dajem, "Prevalence of urinary schistosomiasis and infections with trematode larval stages in *Bulinus truncatus* snails from Qena Upper Egypt" J Appl Sci Res, 2008, vol. 4 (11), pp. 1610-1617.
- [43] G. Schweizer, M.L. Meli, P.R. Torgerson, H. Lutz, P.A. Deplazes, U. Braun, "Prevalence of *Fasciola hepatica* in the intermediate host *Lymnaea truncatula* detected by real time TaqMan PCR in populations from 70 Swiss farms with cattle husbandry" Vet Parasitol, 2007, vol. 150, pp. 164-169.
- [44] D. Singh, P.K. Deepak, "Water quality of Mahesara Lake in Gorakhpur district" Malay J Appl Biol, 2002, vol. 31 (1), pp. 27-30.
- [45] I.K. Jones, A.N. Glazer, "Comparative studies on four sulfhydryl endopeptidases (Ficins) of *Ficus glabrata* latex" J Biol Chem, vol. 245, 1970, pp. 2765- 772.
- [46] R. Giordani, L. Lafon, "Action of *Carica papaya* latex on cell wall glycosidases from *Lactuca sativa*" Phytochem, 1993, vol. 34, pp. 1473-1475.
- [47] K.R. Lynn, "Clevette-Radford NA, Isolation and characterization of proteases from *Euphorbia lacta* and *E. lactea cristata*" Phytochem, 1984, vol. 25, pp. 807-810.
- [48] K. Broglie, I. Chet, M.Holliday, R. Cressman, P. Biddle, "Transgenic plants with enhanced resistance to the fungal pathogen *R. solani*" Science, vol. 245, 1991, pp. 1194-1197.
- [49] S.R. Morcelle, N.O. Caffini, N. Priolo, "Proteolytic properties of *Funarium clausum* latex' Fitoterapia, vol. 75 (5), 2004, pp. 480-493.
- [50] J.R.A. Silva, A.C.F. Amaral, A.C. Siani, C.M. Rezende, J. Felcman, A.C. Pinto, "Contribution to the study of *Himatanthus sucuuba*: latex macromolecule, microelements and carbohydrates" Acta Amaonica, vol. 33 (1), 2003, pp. 105-110.
- [51] F.J. Evans, Naturally occurring phorbol esters, CRC Press, Boca Raton, Florida, 1986.
- [52] A.R. Abdel-monem, E. Abdel-sattar, F.M. Harraz, F. Petereit, "Chemical investigation of *Euphorbia schimperi* C, Presl. Records of Natural Products" vol. 2(2), 2008, pp. 39-45.
- [53] S. Öksüz, F. Gürek, R.R. Gil, T. Pengsuparp, J.M. Pezzuto, G.A. Cordell, "Four diterpene esters from *Euphorbia myrsinites*" Phytochemistry, vol. 38, 1995, pp. 1457-1462.
- [54] A. Falodun, S. Ali, I.M. Quadir, I.M.I. Choudhary, "Phytochemical and biological investigation of chloroform and ethylacetate fractions of *Euphorbia heterophylla* leaf (Euphorbiaceae)" J Med Plants Res, vol. 2(12), 2008, pp. 365-369.