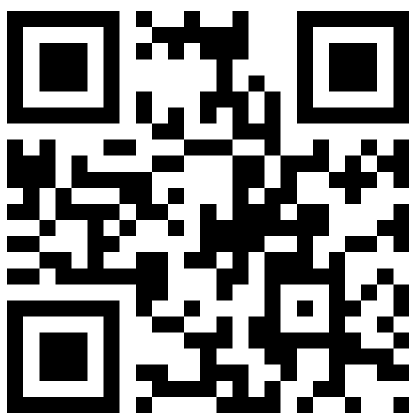
Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Research Article

Received on: 26-03-2017
Accepted on: 01-04-2017
Published on: 15-04-2017

Corresponding Author:

***Jangme C.M.,**
Maharashtra College of Pharmacy,
Nilanga,
Dist. Latur, Maharashtra,
India



*Email Id-
cjangme2011@gmail.com

Qualitative Assessment and Investigation of Antifungal Activity of Methanolic and Petroleum Ether Extracts of *Tectona Grandis* Linn Seeds

Jangme C.M.[1*], Bais S.K.[2], Wadulkar R.D.[3]

ABSTRACT

Tectona grandis has been claimed to be useful in treatment of microbial and fungal infections and the current literature survey revealed that no systematic approach has been made towards documentation of this claim. The currently available synthetic antimicrobial agents are associated with numerous serious side effects like hypersensitivity, crystalluria, photosensitivity, hepatic and renal damage, ototoxicity, neurotoxicity etc. These adverse effects either restrict the continuation of therapy or demand for additional drug/s to combat them. This in turn indicates the need of alternative value addition therapy. In light of this, Methanolic and Petroleum ether extract of *Tectona grandis* was screened for its antifungal activity by using fungal culture but none of extracts were found to be significant in this regard. The claim mentioned in an ancient period may be of value especially in absence of today's effective antifungal agents. As far as today's minimum standard required, it cannot be promoted as antifungal as per these observation.

Key-words: Tectona Grandis, Methanolic and petroleum ether extract, antifungal activity, Hypersensitivity,ototoxicity,Nephrotoxicity.

Cite this article as:
Jangme C.M., Bais S.K., Wadulkar R.D., Qualitative Assessment and Investigation Of Antifungal Activity of Methanolic and Petroleum Ether Extracts of Tectona Grandis Linn Seeds, Asian Journal of Pharmaceutical Technology & Innovation, 05 (23); 13-20, 2017. www.asianpharmtech.com

1Maharashtra College of Pharmacy, Nilanga, Dist. Latur, Maharashtra, India
2Mauli College of Pharmacy, Udgir, Dist. Latur, Maharashtra, India
3Shivlingeshwar College of Pharmacy, Almala, Dist. Latur, Maharashtra, India

INTRODUCTION

Since the introduction of the antibiotics, they have been known as one of the most important tools against fungal infections. Although, in the past years due to the widespread and in some cases incorrect use of antibiotics we can see dramatic increase in microbial resistance against antimicrobial agents that is a critical reason for finding new drugs with less resistance and side effects. The medicinal plants have been used from ancient times especially in Asia, and have been used for the treatment of specific illness.

Over the past 50 years, the development and mass production of chemically synthesized drugs have revolutionized the healthcare system. However, large sections of the population still depend upon traditional practitioners and herbal medicines to meet their health care needs. According to the World Health Organization, 80% of the world's population depends upon traditional medicines for their primary healthcare needs and most of this therapy involves the use of plant extracts and natural components contained therein. A recent shift in the universal trend of synthetic drugs to herbal medicines has been observed even in developed nations. Since the phytochemical constituents responsible for the therapeutic effect present in medicinal plants are also a part of the physiological functions of living flora, it is believed that their better compatibility and tolerability are the leading reasons for their reduced toxicity towards the human body. Hence, herbal medicines have increasingly become the preferred choice especially for common conditions such as cold, sore throat, or bee stings: it is often because professional care is not immediately available, is to inconvenient, costly or time consuming.

Tectona grandis Linn is one such plant whose uses have been claimed and traditionally used to antifungal and alleviate a variety of conditions but comparatively less than adequate scientific validation of these claims has been carried out.

In light of this, the present study titled, "The antifungal activity of Tectona Grandis Linn seeds of mehtanolic and petroleum ether extracts" has been conducted.

Objective

The present work was undertaken to study the Antifungal activity of methanolic and petroleum ether extract of seed of *Tectona grandis* Linn.

Plan of work

The section wise plan of work is given as following:

PHASE – 1

Collection, Identification, Authentication of plant:

Commercially available dry seeds of *Tectona grandis Linn* were purchased in the bulk quantity from local market. The seed were verified for complete dryness and then authenticated by the botanist from the agriculture college, Pune.

Preparation of extracts:

The powder of seeds of *Tectona grandis Linn* were subjected to extraction using methanol and petroleum ether followed by its standardization.

PHASE – 2

Preliminary Phytochemical Analysis of Extracts*:*

The methanolic and petroleum ether extracts were subjected to preliminary phytochemical analysis using standard established methods.

Evaluation of Antifungal activity:

The extracts were screened for antifungal activity using following fungal culture.

Fungal Culture

Candida albicans

Aspergillus niger

Data Analysis and Interpretation

All the results were expressed as Mean ± S.E.M. Data obtained were subjected to statistical analysis using one way ANOVA followed by Dunnett's test using Graph pad Instat software. The results were interpreted using available literature and a conclusion was drawn.

Material and Methods

Preparation of Extracts:

Methanol extract

Tectona grandis dried seeds were charged to extractor along with methanol. It was extracted by heating the mass for 2-3 hours, in a closed system by re-pumping the extract to the herb bed. This process was repeated. The extracts obtained were then combined and filtered. They were then concentrated under vacuum. The extract was then dried in a drier unit and further powdered in a multimill to a fine mesh size. Extract was then sieved using a Sifter to make uniform particle size.

Petroleum ether extract

Tectona grandis dried seeds were charged to extractor along with petroleum ether. It was extracted by heating the mass for 2-3 hours, in a closed system by re-pumping the extract to the herb bed. This process was repeated. The extracts obtained were then combined and filtered. They were then concentrated under vacuum. The extract was then dried in a drier unit and further powdered in a multimill to a fine mesh size. Extract was then sieved using a sifter to make uniform particle size.

Storage of extracts:

Methanolic and petroleum ether extracts of *T. grandis* were stored in tightly closed glass bottles in refrigerator at 2-8^{0}C.

Chemicals and Drugs:

Nutrient agar, Nutrient broth, Saborouds dextrose agar, McFarland solution and Reference standard Griseofulvin. All these drugs and chemicals were purchased either from approved vendors or from the local market as applicable.

Preliminary Phytochemical Analysis of Extracts:

The both extracts were tested for the presence of various chemical constituents in it.

1. Test for Steroids:

a) Salkowaski test:

2 ml chloroform and 2 ml of concentrated sulphuric acid were added to 2 ml of test solution, shaken and allowed to stand. Change in the colour of lower chloroform layer to red and acid layer to greenish yellow fluorescence indicating the presence of steroids was observed.

b) Liebermann-Burchard reaction:

2 ml of test solution was mixed with 2 ml of chloroform. To the solution, 2 ml of acetic anhydride and 2 drops of conc. sulphuric acid from the side of test tube were added. Change in colour first red, then blue and finally green indicating the presence of steroids was observed.

2. Test for Triterpenoids:

a) Salkowaski test:

2 ml of concentrated sulphuric acid () was added to the 2 ml of test solution. This solution was shaken and allowed to stand. Change in the colour of lower layer to yellow indicating presence of triterpenoids was observed.

b) Liebermann-Burchardt Test:

3 ml of test solution was mixed with 3 ml of acetic anhydride. To this, 2 ml of concentrated sulphuric acid was added from the sides of the test-tube. Development of deep red colour indicating the presence of triterpenoids was observed.

3. Test for Glycosides:

a) Balget's test:

2 ml of the test solution was treated with 2 ml of sodium picrate solution and development of yellow to orange colour indicating presence of cardiac glycosides was observed.

b) Keller-Killiani test:

3-5 drops of glacial acetic acid, one drop of 5% $FeCl_3$ and conc. sulphuric acid were added to the test tube containing 2 ml of T.S. Appearance of reddish-brown color at the junction of two layers and bluish green in the upper layer indicating presence of glycosides was observed.

c) Legals test:

To 2 ml of test solution, 1 ml of pyridine and 1 ml of sodium nitroprusside was added. Change in color to pink or red indicating presence of cardiac glycosides was observed.

d) Borntrager's test:

2 ml of dilute sulphuric acid was added to 2 ml of test solution, boiled for few minutes and filtered. To this filtrate, 2 ml of benzene was added and shaken well. The organic layer was separated and ammonia was added. The change in colour of ammonical layer to pink-red indicating presence of anthraquinone glycosides was observed.

e) Tests for Saponin:

1 Foam Test:

20 mg of powdered extract was mixed with 2 ml of water and shaken vigorously. Development of persistent foam which is stable at least for 15 minutes indicating presence of saponin was observed.

4. Tests for Carbohydrates:

a) Molisch's test:

3 ml of Molisch's reagent was added to the 3 ml of test solution and shaken for 3 - 5 minutes. To this, 2 ml of concentrated sulphuric acid was added slowly from the sides of the test tube. The development of a purple ring at the junction of two liquids indicating presence of carbohydrates was observed.

b) Barfoed's test:

1 ml of Barfoed's reagent and 1 ml of test solution were mixed in test tube, heated in boiling water bath for 1-2 minutes and then cooled to room temperature. The appearance of red precipitate indicating the presence of mono-saccharides was observed.

c) Fehling's test:

1 ml of Fehling's A and B solutions were mixed in the test tube and boiled for 1 minute. To this, 2 ml of test solution was added and heated in boiling water bath for the period of 5 - 10 minutes. Appearance of yellow and then brick red precipitate indicating the presence of reducing sugars was observed.

d) Benedict's test:

1 ml of Benedict's reagent and of test solution was mixed in test tube and heated in boiling water bath for 5-10 minutes. Change in colour to yellow, green or red indicating the presence of reducing sugars was observed.

5. Tests for Alkaloids:

To the 20 mg of powder extract, 2 ml of dilute hydrochloric acid was added, shaken well and filtered. With filtrate following tests were performed.

a) Mayer's test:

To the 3 ml of filtrate, 3 drops of Mayer's reagent (potassium mercuric iodide) was added. Appearance of reddish brown or cream precipitate indicating the presence of alkaloids was observed.

b) Hager's test:

4-5 drops of Hager's reagent (saturated picric acid solution) were added to 3 ml of filtrate. Appearance of yellow precipitate indicated presence of alkaloids.

c)Dragendorff's test:

Three ml of the test solution was mixed with Dragendorff's reagent (potassium bismuth iodide). Appearance of reddish brown precipitate indicated presence of alkaloids.

6. Tests for Flavonoids:

a) Ferric-chloride test:

To the test solution, 2 ml of ferric chloride solution were added. The development of intense green colour indicating presence of flavonoids was observed.

b) Shinoda test:

To 10 mg of powdered extract, 5 ml of ethanol (95%), 3 drops of hydrochloric acid and 0.5 gm magnesium turnings were added. Change of colour of solution to pink indicating presence of flavonoids was observed.

7. Tests for Tannins:

a) Ferric-chloride test:

3 ml of test solution was treated with 4-5 drops of ferric chloride solution and development of dark colour indicating presence of tannins was observed.

b) Gelatin test:
Three ml of test solution when treated with gelatin solution (3ml) gave white precipitate.

8. Test for Proteins:
a) Millon's test:
3 ml of test solution and 5 ml of Million's reagent were mixed in a test tube. The appearance of white precipitate changing to brick red which upon heating changes to red indicating presence of proteins was observed.

b) Xanthoproteic test:
To the test tube containing 3 ml of test solution, 1 ml of conc. Sulphuric acidwas added. Appearance of white precipitate which turns yellow on boiling and orange on addition of NH_4OH indicating presence of tyrosin and/or tryptophan containing proteins was observed.
c) Biuret test:
3 ml of the test solution was treated with 4% sodium hydroxide (3 - 5 drops) and 1% copper sulphate solution (3 - 5 drops). The appearance of blue colour indicating presence of proteins was observed.
d) Ninhydrin test:
3 ml of test solution and 3 drops of 5% lead acetate solution were mixed and boiled on water bath for 10 minutes. Change in the colour of solution to purple or blue indicating the presence of amino acids was observed.

9. Test for Fatty Acids:
a) Saponification Test:
The extract was evaporated to get oil. To this oil, 25 ml of 10 % NaOH was added and boiled in boiling water bath for 30 minutes. After cooling, excess Na_2SO_4 solution was added and soap was formed, which rose to the top. This was then filtered. To the filtrate, H_2SO_4 was added and allowed to evaporate. Residue was collected and dissolved in ethanol and thereafter, following tests were performed:
i) To ethanolic solution, few crystals of $KHSO_4$ were added and heated vigorously. Pungent odour of acrylic aldehyde produced indicating presence of fat was observed.
ii) To ethanolic solution, 3 - 5 drops of $CuSO_4$ and NaOH solutions were added. Clear blue solution indicating presence of fat was observed.
Fungal cultures:
The standard pathogenic fungal cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table. 1). The fungi rejuvenated in Sabouraud dextrose (Hi-media laboratories, Mumbai, India) at 37^{0}C for 18 hours and then stocked at 4^{0}C in SDA. Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 ml of sterile Dextrose broth and incubated at 37°C for 3 hours. Turbidity of the culture was standardized to 10^5 CFU with the help of Standard Plate Count (SPC) method and turbid meter.

Table – 1. List offungal cultures used in study

Name of Fungal Strain	MTCC Number
Candida albicans	183
Aspergillus niger	478

Preparation of extracts solutions:
The methanolic (MTG) and petroleum ether (PTG) extracts of *T. grandis* were prepared in distilled water in order to make concentration 100 mg/ml, 200 mg/ml and 400 mg/ml. The doses were administered orally by selecting the appropriate concentration of the stock solution.
Vehicles:
Respective vehicles were prepared by the same procedure without addition of extracts.

Acute toxicity study: (OECD 423, 2001)
Procedure:
Animals were weighed and marked. The acute toxicity of *T. grandis* was evaluated in rats using OECD guideline for testing of chemicals (Acute toxic class method, OECD, 2001).
Healthy adult male albino rats received oral single high dose (2000 mg/kg) of *T. grandis* extract and

were observed for behavioral, neurological and autonomic profiles for the first 4 hour after dosing and periodically for the period of 24 hours for any mortality. Finally, these rats were then maintained for further 14 days with daily observations for any sign of toxicity or mortality.

Evaluation of Antifungal Activity:
Antifungal Activity:
The modified paper disc diffusion (NCCLS, 2000) was employed to determine the antifungal activity of methanolic and petroleum ether extracts of *T. grandis*.For antifungal properties, 0.1 ml culture suspension of 10^5CFU ml-1 was uniformly spread on saborouds dextrose agar (SDA) plate to form lawn cultures. The methanolic and petroleum ether extracts solution were prepared in such a manner that ultimate amount (in dry form) in each disc came to 100mg and 300mg. The blotting paper discs (10mm diameter) were soaked in various diluted extract, dried in oven at 60^0C to remove excess of solvent and tested for their antifungal activity against fungal pathogens by disc diffusion technique. After incubation of 24 hour at 37^0C, zone of inhibition of growth was measured in mm. Griseofulvin 10 mcg (Hi-Media disc) was used as positive control while discs soaked in various organic solvents and dried were placed on lawns as negative control (NCCLS, 2002).

Data Analysis and Interpretation
All the results were expressed as Mean ± S.E.M. Data obtained was subjected to statistical analysis using either student's T-test or one way ANOVA followed by Dunnett's test as per the requirement using Graph pad Instat software. The results were interpreted using published scientific reports and a conclusion was drawn.

RESULTS

Table 2. Preliminary Phytochemical screening of the *Tectona grandis* Linn extract

S.N.	Phytoconstituents	Methanolic extract of *Tectona grandis* Linn (MTG)	Petroleum ether extract of *Tectona grandis* Linn (PTG)
1.	Carbohydrates	-	+
2.	Glycosides	+	+
3.	Saponins	-	+
4.	Flavonoids	+	+
5.	Alkaloids	-	-
6.	Tannins	+	+
7.	Steroids	+	+
8.	Amino Acids	-	-
9.	Proteins	-	-

Acute oral toxicity study (AOT 425)
Oral administration of methanolic and petroleum ether extract of *Tectona grandis* up to the dose of 2000 mg/kg to the respective rats did not show any serious adverse effects or mortality observed continuously for 04 hours and everyday for next 14 days. From this data and pilot study performed at laboratory, three different doses 100, 200 and 400 mg/kg were selected for further study.

Evaluation of antimicrobial activity
Assessment of antifungal activity
Both theextracts *Tectona grandis* Linn did not showed any significant antifungal activity at any dose.

Table No.3. Antifungal activity of MTGagainst fungal pathogens (Zone of inhibition in mm, average of 3 readings)

Fungal Pathogens	Methanolic extract					Negative controls
	10mg /disc	8mg /disc	6mg /disc	4mg /disc	2mg /disc	Methanol
Candida albicans	22	20	19	18	22	-
Aspergillus niger	20	18	17	17	19	-

Table No.4. Antifungal activity of PTGagainst fungal pathogens (Zone of inhibition in mm, average of 3 readings)

Fungal Pathogens	Petroleum ether extract					Negative controls
	10mg /disc	8mg /disc	6mg /disc	4mg /disc	2mg /disc	Petroleum ether
Candida albicans	21	20	18	18	22	-
Aspergillus niger	20	18	16	21	19	-

DISCUSSION

Medicinal plants are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches with lesser side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio friendly plant-based products for the prevention and cure of different human diseases. Considering the adverse effects of synthetic drugs, the world's population is looking for natural remedies which are comparatively safer and equally effective. It is documented that 80% of the world's population has faith in traditional medicine, particularly plant based drugs for their primary healthcare.

Plants consist of numerous constituents called phytochemicals with diverse medicinal values. Moreover, the claims made in the traditional literature are vague and hence scientific validation of these claims is vital in order to make therapy more effective and patient friendly. The present study has been conducted to co-ordinate the traditional claim, experimental observations and role of phytochemicals to establish its close relationship towards the actual therapeutic outcome.

In the present investigation, preliminary phytochemical analysis of methanolic extract of*Tectona grandis*showed the presence of glycosides, flavonoids, tannins, steroids while petroleum ether extract showed the presence of carbohydrates, saponins, glycosides, flavonoids, tannins, steroids. The earlier scientific studies have revealed that these phytochemicals are chiefly responsible for the pharmacological actions and thereby suggested worth to explore the traditional claims. Toxicity is one of the most important aspects of any medication to govern the extent of therapeutic utility. Since preliminary phytochemical results gave indication of further pharmacological screening, it becomes mandatory to evaluate these extracts for their toxicity profile and confirm its safety. As per the principles of general pharmacology, any drug shall not only be pharmacologically effective but also free of toxicity or else its undesirable effect shall be within acceptable range. The maintenance of desirable risk and benefit ratio is prerequisite to label any compound as a drug. The acute oral toxicity studies of methanolic (MTG) and petroleum ether (PTG) extracts of *Tectona grandis* revealed that both these extracts were found to be safe up to the dose of 2000mg/kg. The results of acute oral toxicity study suggested that these extracts can be screened preclinically for validation of its claim in accordance with the previously published reports. Further the pilot study and dose range were found to be effective without any toxic outcome in earlier published reports. Three different doses i.e. 100, 200 and 400

mg/kg for each extract were selected for further preclinical studies (OECD guidelines 421).

Conclusion

Tectona grandis has been claimed to be useful in treatment of microbial and fungal infections and the current literature survey revealed that no systematic approach has been made towards documentation of this claim. The currently available synthetic antimicrobial agents are associated with numerous serious side effects like hypersensitivity, crystalluria, photosensitivity, hepatic and renal damage, ototoxicity, neurotoxicity etc. These adverse effects either restrict the continuation of therapy or demand for additional drug/s to combat them.Thisin turn indicates the need of alternative value addition therapy. In light of this, *Tectona grandis*was screened for its antifungal activity but none of extracts were found to be significant in this regard. The claim mentioned in an ancient period may be of value especially in absence of today's effective antimicrobial agents. As far as today's minimum standard required, it cannot be promoted as antifungal as per these observation and for to use clinical further study is required.

.

REFERENCES

1. Adegeye AO, Ogunsanwo OY. and Olajuyigbe SO. Antifungal Activities of Heart Wood Extract (HWE) of Teak, *Tectona Grandis* against two white rots in woods of *Gmelina arborea* and *Triplochitonscleroxylon.* Academic Journal of Plant Sciences, 2009; 2(4),.279-285.
2. Aebi, H., Catalase. In: Bergmeyer, H.V. (Ed.), Methods in Enzymatic Analysis, vol. 2. Academic Press, New York, Chemie, Weinheim, FRG, 1974; 674–684
3. Sanjoy Kumar Pal, Yogeshwer Shukla , Herbal Medicine: Current Status and the Future Asian Pacific Journal of Cancer Prevention, 2003;4.
4. Kamboj VP, Herbal medicine, Current Science, 2000; 78(1):10.
5. Kopa Theodora K, Tchinda Alembert T, MichelF. Tala, Denis Zofou, Ransom Jumbam, Hippolyte K. Wabo, Vincent PK. Titanji, Michel Frédérich, Ning-Hua Tan, Pierre Tane, Antiplasmodial anthraquinones & hemisynthetic derivatives from the leaves of *Tectona grandis* (Verbenaceae), *Phytochemistry Letters, 2014.*
6. Bhattacharjee Supriya Kumar. *Handbook of medicinal plants.*: Aavishkar Publishers;2000.
7. Binder, HJ., Dobbins, JW., Racusen, LC., Whiting, DS. Effectof propanolol on ricinoleic acid and deoxycholic acid-induced changes of intestinal electrolyte involvement and mucosal permeability. Gastroenterology, 1978;75,668–673.
8. Bermudez, M., Hazen, T.C. Phenotypic and genotypic comparision of *Escherichia Coli* from pristine tropical waters Applied and environmetsl Microbiology. 1988;58,.979-983.
9. Patkar Atul N, Desai Nilesh V.Range Akkatai A, Kalekaekar Kamlakar S., A review of Algle mormelos: A potential medicianal tree, International research Journal of Pharmacy, 2012; 3:8.
10. Rajaram S. Sawant and Ashvin G. Godghate, Preliminary phytochemical analysis of leaves of tridax procumbens linn, International Journal of Science, Environment, and Technology, Vol. 2, No 3, 2013, 388–39
11. Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. *J Adv Pharm Technol Res.*2011;2(2):104–9.
12. Aguinaldo AM., Osler PM., Ocampo BF., Bowden AI., Peter G. Tectograndone, an anthraquinone naphthoquinone pigment from the leaves of Tectona. Phytochemistry. 1993; 33(4):933-5.
13. Alam, AHMK., R. Islam, KA. Salam, MM. Manir, MA. Baki, MA. Hossain and G. Sadik, 2006. Toxicological studies of N-transferloyl- 4methyldopamine isolated from *Achranthes ferruginea*. Pakistan Journal of Biological Sciences, 9:1052-1055.
14. Anisuzzaman, ASM., N. Sugimoto, G. Sadik andM.A. Gufor, 2001. Sub-acute toxicity study of 5- hydroxy-2(hydroxy-methyl) 4H-pyran-4- One, isolated from *Aspergillus fumigatus*. Pakistan Journal of Biological Sciences,1012-1015.
15. Ann-Mari, Svennerholm, 2011. From cholera to enterotoxigenic *Escherichia coli* (ETEC)vaccine development. Indian J Med Res. 133 (2),188 - 194
16. Araujo, JM, Tabarelli KR. Aranda SH. Fabbricotti, Fagundes Neto U, and Scaletsky IC. Typical enteroaggregative and atypical enteropathogenic types of Escherichia coli are the most prevalent diarrheaassociated pathotypes among Brazilian children. J. Clin. Microbiol. 2007;45,3396–3399.
17. Balassiano IT., *et al*. Demonstration of the lapachol as a potential drug for reducing cancer metastasis. *Oncol. Rep.*, 2005; 13(2):329-33.
18. Cookson, ST., and Nataro, JP. Characterization of HEp-2 cell projection formation induced by diffusely adherent *Escherichia coli*. Microb. Pathog. 1996;21:421-434.