Research Article
Available online at www.ijpras.com

ISSN 2277-3657

Volume 3, issue 2 (2014),24-29

International Journal of Pharmaceutical Research & Allied Sciences

Antibacterial Activity of *Euphorbia hirta* L. Ethanomedicinal Plant against Gram Negative UTI Pathogens

Nikunj B. Patel* [1], Kaushik C. Patel [2]
Smt. S. S. Patel Nootan Science and Commerce College, Visnagar, Gujarat, India[1]
Smt. S. M. Panchal Science College, Talod, Gujarat, India [2]
*niks17micro@gmail.com

Subject:Microbiology

Abstract

Young branches with leaves and inflorescence part of *Euphorbia hirta* used in traditional medicine for the therapeutic purpose was extracted by aqueous, petroleum ether, acetone and methanol. The disc diffusion method was used to determine the antibacterial activity on gram negative: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Klebsiella pneumoniae* UTI pathogens.The results indicated that the petroleum ether extract of *Euphorbia hirta* showed high zone of inhibition against gram negative organism *P. vulgaris* (21.67 mm), aqueous extract showed high zone of inhibition against *P. aeruginosa* (15 mm), methanol extract showed high zone of inhibition against *E. coli* (14 mm), whereas The minimum inhibitory concentration (MIC) for acetone, aqueous and petroleum ether extracts of *Euphorbia hirta* plant displayed an excellent antibacterial activity against *K. pneumoniae* with MIC value 12.5µg/µl, methanol extract displayed an excellent antibacterial activity against *P. aeruginosa* and *K. pneumoniae* with MIC value 12.5µg/µl.

Key Words: *Euphorbia hirta*, *Antibacterial*, *Disc diffusion*, *Minimum Inhibitory Concentration.*

Introduction

Euphorbia hirta L. is belongs to family *Euphorbiaceae*, commonly known as 'Rati - Dudheli'. It is an annual hairy plant. It is rich in waste places over the roadsides and also available open grasslands. The *E. hirta* have been documented to be able to include saponins, alkaloids, flavonoids, tannins phenolic acids and amino acids.[1, 2, 3] It can grow to a height of 40 cm. The stem is slender and frequently reddish in color, covered with yellowish bristly hairs specifically in the younger parts. The actual leaves tend to be oppositely arranged, lanceolate and are usually greenish or reddish underneath measuring about 5 cm long. Within the axils appear really very small dense round clusters of flowers. The small green flowers constitute the inflorescence characteristic of the euphobias. The stem and leaves produce white or milky juice when cut.[4]

Traditionally, Plant is use within conjunctivitis, exhibits antipyretic, anthelmintic, antispasmodic, antibacterial, antifertility, antifungal, and antiinflammatory activities.[5, 6] Our literature survey revealed that there is no experimental evidence of antidiabetic effect of the plant. Therefore, the ethanolic and petroleum ether flower extracts of *E. hirta* influences lipid parameters in alloxan induced diabetic mice.[7] Molecular continues to be concerned within humanillness including coronary heart failure, nephrotoxicity, lung illness, inflammation and also diabetes.[3, 8]

Materials & Methods

Ethno medicinal Survey

The collections of ethnobotanical plants through well-planned explorations in various range forest areas of North Gujarat like; Taranga (Mehsana district), Danta (Banaskantha district), Ambaji (Banaskantha district), Jassore (Banaskantha district), Vireshwar (Sabarkantha district), Vijaynagar (Sabarkantha district).

Collection, Identification and Authentication of the Plants

In various seasons, various ethnomedicinal useful plant parts like; root, stem, bark, leaves, flowers and fruits were collected fresh/dry for laboratory work. Selected wild medicinal plant species were identified with dissecting floral parts (in the field/laboratory) and with the help of regional floras for scientific justification.

All the specimens were critically examined in the laboratory under light microscope and identified with the help of the state and/or the regional floras and other authentic literature on taxonomy available in the College / University library. The identification was finally confirmed by matching with the help of authentic herbarium specimens available at S. P. University Herbarium, Vallabh Vidyanagar (Gujarat) and Saxton & Sedgwick's collection available at Gujarat College, Ahmedabad.

Extraction of Plant Materials

Young branches with leaves and inflorescence of *Euphorbia hirta* (*Euphorbiaceae*) was washed thoroughly under running tap water and dried on paper towel, then kept in room temperature for proper drying and finally powdered.

10 gm of air dried plant powder (leaves, stem, fruits, etc.) is successively with 100 ml of aqueous and organic solvents petroleum ether, acetone and methanol separately in 250 ml sterile conical flask and covered with cotton wool. It was then plugged and wrapped with aluminum foil and shaken vigorously for 24 hrs. at room temperature. The mixture was then filtered using a Whatman No. 1 filter paper. The filtrate was evaporated at 50-55ºC on a water bath in reflux condition to obtain crude extract. Extraction procedure was done further twice for complete extraction. Then the crude extracts were resuspended in the respective solvents to prepare various concentrations of 100, 50, 25, 12.5µg/µl before testing for antibacterial activity. The same procedure was followed for all solvent extraction.[9, 10, 11, 12]

Bacterial Cultures

The following bacteria were employed in the screening: Gram- negative: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Klebsiella pneumoniae* were isolated from hospitalized patients from the A. M. C. M. E. T College, L. G Hospital Compound, Ahmadabad (North Gujarat) and its identify confirmed by biochemical tests. The stock cultures were maintained at 4°C on slopes of Nutrient agar and sub cultured for 24 hrs before use. [12, 13]

Antibiotics

Hi-media antibiotics used in the study were Amoxicillin (30 mcg), Bacitracin (10 units), Ciprofloxacin (5 mcg), Gentamicin (10 mcg), Nalidixic Acid (30 mcg), Penicillin-G (10 units), Streptomycin (10 mcg), Tetracycline (30 mcg).

Preparation of inoculums

Direct colony suspension method of choice for UTI organisms, e.g. *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Klebsiella pneumoniae* colonies are taken directly from the plate into distilled water. The suspension should match or exceed the density of the 0.5 McFarland standard. These suspensions should be used within 30 min of preparation.[14]

Antibacterial Sensitivity Testing

Kirby-Bauer Disc Diffusion Method

Antibacterial activity of the aqueous, petroleum ether, acetone and methanol extracts were determined using the agar disc diffusion by Kirby-Bauer method.[15, 16] Kirby-Bauer method is recommended by the National Committee for Clinical Laboratory Standards (1993) and the World Health Organization (WHO). Sterile Petri plates containing Mueller-Hinton agar (Hi-media) used for the assays and 100 µl standardized inoculum (which has been adjusted to 0.5 McFarland standard), was spread using a sterile glass spreader by spread plate method. 6mm diameter of sterile Whatman® No.1 filter paper discs was prepared. Negative control was prepared using respective solvent, while the standard Hi-media antibiotic discs served as a positive control were aseptically placed over sterile Mueller-Hinton agar plates seeded with respective test organisms. 100 µg/µl of crude extract were aseptically transferred to these discs. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 min at 37˚C. The plates were incubated inverted position at 37˚C or 24 hrs and each extract was tested on three replicate plates. At the end of incubation inhibition zone formed around the disc were measured in mm (millimeter) and the results were recorded.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibition Concentrations (MIC's) was determined using Inhibitory Concentrations in Diffusion (ICD) method.[17, 18] The MIC method was applied on extracts that proved their high efficacy against m.o. The highest dilution of a plant extract that still retain an inhibitory effect against the growth of an m.o. is known as MIC. Minimum Inhibitory Concentration (MIC) was carried out with Whatman® No.1 filter paper was impregnated with various concentration (100, 50, 25, 12.5 µg/µl) of extracts prepared using respective solvent were placed on Mueller-Hinton agar plates. 100 µl standardized inoculum 0.5 McFarland standards was spread using a sterile glass spreader by spread plate method. All the plates were incubated at 37˚C for 24 hrs. After 24 hrs, the zone of inhibition appearing around the discs was measured in each concentration and recorded in millimeter diameter. The lowest concentration of the

extracts which inhibits the growth of tested bacteria is observed.

Statistical analysis
All the tests were conducted in triplicates. The data of all the parameters were statistically analyzed and expressed as mean ± S.D.

Results and Discussion

Antibacterial Sensitivity Testing

Kirby-Bauer Disc Diffusion Method
The significant antibacterial activities of the gram positive and negative organisms were comparable to the standard Hi-media antibiotic discs by Kirby-Bauer disc diffusion method. The results show the antibiotic sensitivity testing against the gram negative microorganisms. **(Fig.1)**

On the other hand *E. coli* gram negative bacterium was sensitive to Amoxicillin, Bacitracin, Tetracycline and Ciprofloxacin. Second gram negative bacterium *P. aeruginosa* was sensitive to Gentamicin and Ciprofloxacin. Third gram negative bacterium *P. vulgaris* was sensitive to Streptomycin and resistant to Ciprofloxacin. Fourth gram negative bacterium *K. pneumoniae* was sensitive to Gentamicin and Streptomycin, whereas showed intermediate to Ciprofloxacin.

The isolated bacterial Urinary Tract Infection (UTI) pathogens were identified based on the morphological and biochemical characteristics. The extracts were tested for the antibacterial activity against the UTI pathogens. The acetone, methanol, Aqueous and petroleum ether extracts of *Euphorbia hirta* medicinal plant showed inhibitory effect against Gram negative: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumoniae* UTI pathogens. **(Table. 1)**

The acetone extract of *Euphorbia hirta* showed zone of inhibition against gram negative organism *K. pneumoniae* followed by respectively *P. aeruginosa*, *E. coli* and *P. vulgaris.* **(Table.1)**

The methanol extract of *Euphorbia hirta* showed zone of inhibition against gram negative organism *K. pneumoniae* followed by respectively *E. coli*, *P. aeruginosa* and *P. vulgaris.* **(Table.1)**

The aqueous extract of *Euphorbia hirta* showed zone of inhibition against gram negative organism *K. pneumoniae* followed by respectively *P. aeruginosa*, *E. coli* and *P. vulgaris.* **(Table.1)** The petroleum ether extract of *Euphorbia hirta* showed zone of inhibition against gram negative organism *P. vulgaris* followed by *K. pneumoniae.* Surprisingly, no inhibitory effect has been noted for gram negative, *E. coli* and *P. aeruginosa* organism. **(Table.1)**

Determination of Minimum Inhibitory Concentration (MIC)

If plant extract displayed an MIC 12.5µg/µl, the antibacterial activity was considered as excellent. The MIC value 25, 50 and 100µg/µl, the antibacterial activity was considered as respectively good, moderate and weak. Similarly, the MIC value over 100µg/µl, the antibacterial activity was considered as inactive.

The acetone extract of *Euphorbia hirta* plant displayed an excellent antibacterial activity against *K. pneumoniae* with MIC value 12.5µg/µl, but were moderate antibacterial activity against *E.coli*, *P. aeruginosa* and *P. vulgaris* with MIC value 25µg/µl. **(Fig.2)**

The methanol extract of *Euphorbia hirta* plant displayed an excellent antibacterial activity against *P. aeruginosa* and *K. pneumoniae* with MIC value 12.5µg/µl, but were good antibacterial activity against *E.coli* with MIC value 25µg/µl, while a moderate antibacterial activity against *P. vulgaris* with MIC value 50µg/µl. **(Fig.2)**

The aqueous extract of *Euphorbia hirta* plant displayed an excellent antibacterial activity against *K. pneumoniae* with MIC value 12.5µg/µl, but was good antibacterial activity against *P. aeruginosa* with MIC value 25µg/µl, while a moderate antibacterial activity against *E. coli* with MIC value 50µg/µl, when a weak antibacterial activity against *P. vulgaris* with MIC value 100µg/µl. **(Fig.2)**

The petroleum ether extract of *Euphorbia hirta* plant displayed a good antibacterial activity against *K. pneumoniae* with MIC value 25µg/µl, but were a moderate antibacterial activity against *P. vulgaris* with MIC value 50µg/µl. **(Fig.2)**

Discussion

The ethanolic and petroleum ether flower extracts of *Euphorbia hirta* influences lipid parameters in alloxan induced diabetic mice.[7] The preponderance of saponin in the extracts of *E. hirta* that reported by previous researchers could then justify the use of these plants in the treatment of some microbial

infections mentioned earlier. [19, 20, 21] The results obtained indicated that the ethanolic extract of the *Euphorbia hirta* plant inhibited the growth of the test isolates except *Salmonella typhi.* This therefore shows that the extract contains substance(s) that can inhibit the growth of some microorganisms. Other workers have also shown that extracts of plants inhibit the growth of various microorganisms at different concentrations.[22, 23, 24, 25, 26] The observed antibacterial effects on the isolates is believed to be due to the presence of alkaloids, tannins and flavonoids which have been shown to posses antibacterial properties.[27, 28]

Some workers have also attributed their observed antimicrobial effects of plant extracts to the presence of these secondary metabolites.[24] Some workers have also identified tannins, flavonoids and alkaloids in the extracts of the plant.[29, 30] The large zones of inhibition exhibited by the extract on *S. aureus* and *P. aeruginosa* justified their use by traditional medical practitioners in the treatment of sores, bores and open wounds. *S. aureus* and *P. aeruginosa* have been implicated in cases of boils, sores and wounds.[31] Also the moderate growth inhibition on *E. coli* justifies its use in the control of diarrhoea and dysentery. *E. coli* is the common cause of traveler's diarrhoea and other diarrhoeagenic infections in humans. [31] The low MIC exhibited by the extract on *S. aureus* is of great significance in the health care delivery system, since it could be used as an alternative to orthodox antibiotics in the treatment of infections due to this microorganism, especially as they frequently develop resistance to known antibiotics. [31] Their use also will reduce the cost of obtaining health care. The relatively high zone of inhibition exhibited by the extract on *E. coli* is also of significance, since *E. coli* is a common cause of diarrhea in developing countries. [31]

Conclusion

In conclusion, the results of the *in vitro* study showed by performing the consensus scoring, we have reported that aqueous extract of *Euphorbia hirta* L. medicinal plants possessed high inhibitory activity and low MIC followed by respectively methanol, acetone and petroleum ether extracts for gram negative bacteria UTI pathogens, confirming the great potential of these plants used in North Gujarat ethnomedicine for the production of bioactive compounds and useful for this medicinal plant in primary health care.

Table.1 Antibacterial activity of acetone, methanol, aqueous and petroleum ether extracts of *Euphorbia hirta* L. medicinal plant against UTI pathogens (diameter of inhibition zone in mm*)

Euphorbia hirta	Solvent	*E. coli*	*P. aeruginosa*	*P. vulgaris*	*Klebsiella pneumoniae*
	Acetone	8.67±1.53	12.67±0.58	8.33±0.58	13.00+1.00
	Methanol	14.00±1.73	12.67±0.58	11.00±1.00	16.00+1.00
	Aqueous	13.00±1.00	15.00±1.00	9.00±1.00	16.67+0.58
	Petroleum Ether	-	-	21.67±0.58	15.67+0.58

* The values are mean zone of inhibition (mm)± S.D. of three replicates.

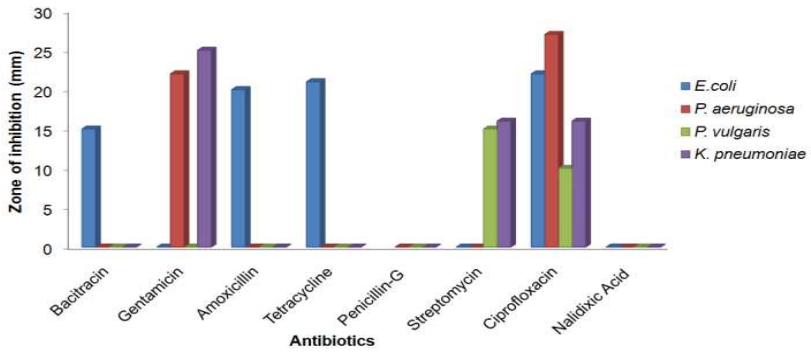

Fig.1 Inhibitory effects of UTI Pathogens on Standard Antibiotics

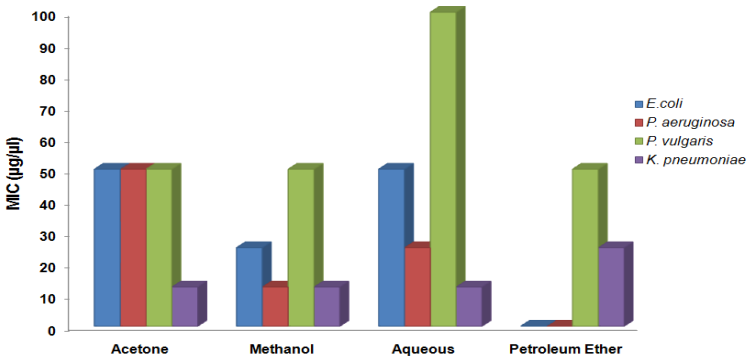

Fig. 2 Minimum Inhibitory Concentration (MIC) (µg/µl) of Acetone, Methanol, Aqueous and Petroleum Ether extracts from *Euphorbia hirta* Medicinal Plant from North Gujarat against UTI Pathogens

"Cite this Article"

N. B. Patel, K. C. Patel "Antibacterial Activity of Euphorbia hirta L. Ethanomedicinal Plant against Gram Negative UTI Pathogens" Int. J. of Pharm. Res. & All. Sci.2014;3(2),24-29

References

1. Hore, S. K., Ahuja, V., Mehta, G., Kumar, P., Pandey, S. K., Ahmad, A. H., Effect of aqueous *Euphorbia hirta* leaf extract on gastrointestinal motility, Fitoterapia, 2006, 77, 35-38.
2. Mangathayaru, K., Sravan, K., Reddy, P. K., Kumar, M., Sweta, B., Reddy, U. C., *In vitro* antioxidant studies on the aerial parts of Origanum *majoram* Linn. and *Artemesia sieversiana* earh, Pharmacognosy Magazine, 2007, 3 (10), 90-94.
3. Kandalkar, A., Patel, A., Darade, S., Baviskar, D., Free radical scavenging activity of *Euphorbia hirta* linn. leaves and Isolation of active flavonoid myricitrin, asian Jour. of pharma. and clinical Res., 2010, 3(3), 234-237.
4. Lind, E. M., Tallantire, A. C., Some Common Flowering Plants of Uganda, Oxford University Press, Nairobi, 1971, 182.
5. Sood, S. K., Bhardwaj, R., Lakhanpal, T. N., Ethnic Indian Plants in cure of diabetes, Scientific Publishers, Jodhpur, India,2005, 64-65.
6. Elizabeth, M. W., Major Herbs of Ayurveda, Churchill Livingstone, China, 2002, 64-65.
7. Kumar, S., Malhotra, R., Kumar, D., Antidiabetic and Free Radicals Scavenging Potential of *Euphorbia hirta* Flower Extract, Indian J. Pharm. Sci., 2010, 72(4), 533-537.
8. Gopinathan, N., Shrinivasan, K. K., Mathew, J. E., Free radical scavenging scavenging properties of the ethanol extract of *Saccharum spmtaneum*, Indian drugs, 2004, 41(10), 633-635.
9. Omogbai, B. A., Eze, F. A., Phytochemical Screening and Susceptibility of Bacteria Pathogens to Extracts of *Evolvulus alsinoides*, Sci. World Jour., 2011, 6 (1), 5-8.
10. Kokate, C. K., Purohit, A. P., Gokhle, S. B., Pharmacognosy, Nirali Prakashan, Pune, 2007, 37, 105-108.
11. Akharaiyi, F. C., Bolatito, B., Antibacterial and Phytochemical Evaluation of Three Medicinal Plants, Jour. of Natural Products, 2010, 3, 27-34.
12. Sundaram, S., Dwivedi, P., Purwar, S., Antibacterial Activities of Crude Extracts of *Chlorophytum borivilianum* to Bacterial Pathogens, Res. Jour. of Medi. Plant, 2011, 5, 343-347.
13. Ghosh, A., Das, B. K., Roy, A., Mandal, B., Chandra, G., Antibacterial activity of some medicinal plant extracts, J. Nat. Med., 2008, 62, 259-262.
14. Andrews J. M., Determination of minimum inhibitory concentration, Jour. of Antimicrobial Chemotherapy, 2001, 48 (S1), 5-16.
15. Baur, A. W., Kirby, W. M., Sherris, J. C, Turck, M., Antibiotic susceptibility testing by a standard single disk method, Am. J. Clin. Path., 1966, 45, 493-496.
16. Mohamed, S. S. H., Hansi, P. D., Kavitha, T., Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants, Inter. Jour. of Pharma. Sci. and Res. (IJPSR), 2010, 1(10), 430-434.

17. Guerin-Faublee, V., Muller, M. L. D., Vigneulle, M., Flandrois, J. P., Application of a modified disc diffusion technique to antimicrobial susceptibility testing of *Vibrio anguillarum* and *Aeromonas salmonicida* clinical isolates, Veterinary Micro., 1996, 51, 137-149.
18. Zaidan, M. R. S., Rain, N. A., Badrul, A. R., Adlin, A., Norazah, A., Zakiah, I., *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method, Tropical Biomedicine, 2005, 22(2), 165-170.
19. Johnson, P. B., Abdurahman, E. M., Tiam, E. A., Abdu-Aguye, I., Hussaini, I. M., *Euphorbia hirta* leaf extracts increase urine output and electrolytes in rats, J. Ethnopharm., 1999, 65, 63-69.
20. Tona, L., Cimanga, R. K., Mesia, K., Musamba, C. T., Bruyne, T., *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the democratic republic of Congo. J. Ethnopharm., 2004, 93, 27-32.
21. Sudhakar, M., Rao, C. V., Rao, P. M., Raju, D. B., Venkateswarlu, Y., Antimicrobial activity of *Caesalpinia pulcherrima*, *Euphorbia hirta*. and *Asystasia gangeticum*, J. Fitoterpia, 2006, 77, 378-380.
22. Akujobi, C., Anyanwu, B. N., Onyeze, C., Ibekwe, V. I., Antibacterial Activities and Preliminary Phytochemical Screening of Four Medicinal Plants, Jour. of Applied Sci., 2004, 7(3), 4328-4338.
23. Esimone, C. O., Adiukwu, M. U., Okonta, J. M., Preliminary Antimicrobial Screening of the Ethanolic Extract from the Lichen Usnea subfloridans (L), Jour. of Pharma. Res. and Dev., 1998, 3(2), 99-102.
24. Nweze, E. I., Okafor, J. I., Njoku, O., Antimicrobial activities of methanolic extracts of *Trema guineensis* (Schumm and Thorn) and *Morinda lucida* Benth used in Nigerian Herbal Medicinal Practice, Jour. of Biological Res. and Biotech., 2004, 2(1), 39-46.
25. Ntiejumokwu, S., Alemika, T. O. E., Antimicrobial and Phytochemical Investigation of the Stem bark of *Boswellia dalziella*, West African Jour. of Pharmacology and Drug Res., 1991, 10, 100-104.
26. Osadebe, P. O., Ukwueze, S. E., A Comparative Study of the Phytochemical and Antimicrobial Properties of the Eastern Nigerian Species of African Mistletoe (*Loranthus micranthus*) sourced from different host trees, Jour. of Biological Res. and Biotech., 2004, 2(1), 18-23.
27. Cowan, M. M., Plant Products as Antimicrobial Agents, Clinical Micro. Review, 1999, 12, 564-582.
28. Draughon, F. A., Use of Botanicals as Biopreservatives in Foods, Food Tech., 2004, 58(2), 20-28.
29. Yoshida, T., Namba, O., Chen, L., Okuda, T., Euphorbin E, a hydrolysable tannin dimmer of highly oxidized structure from *Euphorbia hirta*, Chem. Pharm. Bull, 1990, 38(4), 1113-1115.
30. Blanc, P., Sacqui-Sonnes, G., Flavonoids of *E. hirta*, Plant Med. Phytother., 1972, 6, 106-109.
31. Ogueke, C. C., Ogbulie, J. N., Okoli, I. C., Anyanwu, B. N., Antibacterial Activities and Toxicological Potentials of Crude Ethanolic Extracts of *Euphorbia hirta*, Jour. of American Sci., 2007, 3(3), 11-16.