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Full Length Research Paper

In vitro pharmacological activity of the crude acetone extract of *Erythrina caffra* Thunb: antibacterial and antifungal assessment

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The antimicrobial activity of stem bark acetone extract of *Erythrina caffra* Thunb. against medically important pathogens was evaluated by determining the inhibition zones and the minimum inhibitory concentrations (MICs) of the extract. The result showed that the extract had good antimicrobial activity against the tested bacterial and fungal isolates with the increases in the inhibition zones being concentration dependent. The degree of the antibacterial activity indicated that the minimum inhibitory concentration (MIC) values ranged between 0.0195 and 1.25 mg/ml while the minimum bactericidal concentrations (MBC) ranged between 0.0391 and 1.25 mg/ml for the extract. The extract was less potent than the standard antibiotic, ciprofloxacin, having MIC values of (0.0195 to 0.625 µg/ml). For the fungal isolates, the MIC and minimum fungicidal concentration (MFC) values ranged between 0.625 and 20 mg/ml. Though the results showed that the bacteria were more susceptible to the extract than the fungi, the extract inhibited Gram-negative bacteria more than the Gram-positive bacteria at low concentrations. This study shows a broad spectrum and great therapeutic potential of the plant with the ability to attract significant scientific attention.

Key words: Antimicrobial activity, broad spectrum, crude extract, fungicidal concentration, inhibitory effects, minimum inhibitory concentration (MIC) test.

INTRODUCTION

Traditional health care delivery of which plants have played significant roles and antimicrobial properties of plant-derived compounds in most parts of the world have been well documented (Zuo et al., 2008; Chomnawang et al., 2009; Olajuyigbe and Afolayan, 2011a). In virtually all cultures, medicinal plants have been used as sources of medicines (Anwannil and Atta, 2006). In developing countries, where the use of medicinal plants in treating diseases is an ancient tradition, herbal medicines have formed a significant part of culture and traditions of the rural people. Here, folk medicinal practitioners are the primary health-care providers (Rahmatullah et al., 2010). Globally, more than 80% of the population in developing countries of the world (Eddouks et al., 2002) out of about 64% of the total population remains dependent on traditional medicine and medicinal plants for provision of their health-care need (Cotton, 1996). In South Africa, many people prefer plants as an alternative or supplement medicines to visiting a western health care practitioner (Van Wyk et al., 1997). They rely on medicinal plants because of their continued availability, effectiveness, lack of modern healthcare alternatives and cultural preferences (Caniago and Siebert, 1998), better compatibility (Kamboj, 2000) and high acceptance (Ghimire et al., 2005) as well as high cost of developing patentable chemicals and drugs (Hack, 2006).

Although plant-based traditional knowledge has become a recognized tool in search for new sources of drugs and neutraceuticals (Gosh, 2003), folk medicine is gradually losing its attraction among the young generation who are depending on western medicine (Lin, 2005).

The therapeutic efficacies of medicinal plant are often criticized due to deficiency of research, critical evaluation

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and validations to support the safety of uses (Houghton, 1995; Fong, 2002). While a lot of traditional medicines have been reported with different pharmacological actions (Gregory, 2004), the increasing use of traditional therapies, however, provides scientifically sound evidences for the principles behind therapies and for effectiveness of plant medicines (Patwardhan et al., 2005). With extensive research on pharmacological importance and uses of medicinal plants in the treatments of infections, numerous drugs have been introduced into the international market through exploration of ethnopharmacology and traditional medicine (Bussmann, 2002). Consequently, a consideration for medicinal plants as a significant source of potentially pharmacologically important phytochemicals has become inestimable.

Erythrina is a genus of flowering plants in the pea family, Fabaceae (SWGB, 1995). It consists of about 130 species distributed in the tropical and subtropical regions of the world. They are often cultivated as an ornamental tree, street and park trees in drier areas as well as shade and soil improvement tree fixing nitrogen for other tree crops such as coffee and cacao (Teketay, 1990; Fassbender et al., 1991).

In traditional medicine, the genus has been prominently used in folk medicine (Tanaka et al., 1999; Chawla and Kapoor, 1995), especially for the treatment of microbial infections (Mitscher et al., 1987). The seeds of at least one-third of the species contain potent erythrina alkaloids, and some of these are used for medicinal and other purposes by indigenous peoples. While a number of species of the genus *Erythrina* are rich in secondary metabolites, particularly flavonoids, phenolics and alkaloids that exhibit interesting anti-inflammatory, anti-plasmodial, bactericidal, curariform and fungicidal activities, many of these plants have a significant history of medicinal use for the treatment of diseases such as female infertility, stomach pain and gonorrhea (Irvine, 1961).

Erythrina caffra Thunb., the Coast Coral Tree, is a tree native to southeastern Africa. It is widely loved for its warm red to scarlet-coloured flowers which appear from the cold winter months up to spring. It occurs in the warm and frost-free to light frost coastal regions of the Eastern Cape and northern KwaZulu-Natal. The bark of *E. caffra* is used to treat sores, tuberculosis, respiratory infections, wounds, abscesses, arthritis and toothache. The infusions of the leaves are used as eardrops for earache while the decoctions of the roots are used for sprains (Roberts, 1990; Hutchings et al., 1996). *E. caffra* is a plant of medicinal importance for which there is a dearth of scientific reports.

In this vein, since the systematic screening of medicinal plant extracts for antimicrobial activities represents a continuous effort to finding new compounds with potential to act against pathogenic bacteria and fungi, this study was aimed at investigating the antimicrobial potential of the acetone bark extract of *E. caffra* in order to validate its relevance in the treatment of microbial infections by the rural communities.

MATERIALS AND METHODS

Collection of plant material

The bark materials of *E. caffra* were collected in September, 2010, from the plant growing within the University of Fort Hare campus in Alice, South Africa. The plant was authenticated in the Department of Botany and a voucher specimen was prepared and deposited in the Griffen Herbarium of the University.

Extract preparation

The bark sample was air-dried at room temperature and pulverized using a milling machine. About 100 g of the pulverized sample was extracted with 500 ml of acetone for 72 h. The extract was filtered through Whatman No. 1 filter paper and evaporated to dryness under reduced pressure at 40°C using a rotary evaporator. A know amount of the crude extract was reconstituted in 100% dimethyl sulfoxide (DMSO) to make 100 mg/ml stock (w/v). Different working stocks of the extract in DMSO were prepared for the bioassay analysis. Both stocks were store at 4°C until further use for evaluations.

The reconstituted extract solution in DMSO was sterilized by filtering through 0.45 µm membrane filter and tested for sterility after membrane filtration by introducing 2 ml of the extract into 10 ml of sterile nutrient broth and incubated at 37°C for 24 h. A sterile extract was indicated by the absence of turbidity in the broth after the incubation period (Ronald, 1995).

Test organisms

The bacteria used in this study included *Streptococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 4352, *Serratia mercescences* ATCC 9986, *K. pneumoniae* ATCC 10031, *Enterococcus cloacae* ATCC 13047, *Proteus vulgaris* ATCC 6830, *Shigella sonnei* ATCC 29930, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 19582, *K. pneumoniae* KZN, *Bacillus subtilis* KZN, *Shigella flexneri* KZN, *Staphylococcus aureus* OK_3, *S. aureus* OK_{2a}, *S. aureus* OK_1 and *Acinetobacter calcaoceuticus anitratis* CSIR. The fungi included *Candida krusei*, *Candida albicans*, *Candida neoformans*, *Candida rugosa*, *Penicillium notatum*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus*, *Absidia corymbifera*, *Candida glabrata*, *Trichophyton mucoides*, *Trichophyton tonsurans* and *Fusarium sporotrichioides*. These organisms were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa. The antibacterial assays were carried out using Mueller Hinton II Agar (Biolab) and broth. The antifungal assays were carried out using sabouraud dextrose broth and potato dextrose agar.

Antimicrobial assay by agar diffusion method (Inhibition zones)

The susceptibility screening of the test bacteria to the acetone bark extract of *E. caffra* and ciprofloxacin, used as control, was done in accordance with the methods described by Irobi et al. (1994) and Akinpelu et al. (2008). For the antibacterial assay, the inoculum of each test bacterial strains was standardized at 5×10^6 cfu/ml using McFarland Nephe-lometer standard (NCCLS, 1993). Sterile Mueller Hinton agar plates were seeded with each adjusted test bacterial

strain and allowed to stand at 37℃ for 30 min. Wells were then bored into the agar medium with heat sterilized 6 mm cork borer. The wells were filled with 100 µl of different concentrations (5, 10 and 20 mg/ml) of the extract and ciprofloxacin (1.25, 2.5 and 5 µg/ml) taking care not to allow spillage of the solutions onto the surface of the agar.

For the antifungal assay, 1 cm^2 of seven day old fungal cultures was dropped in sterile distilled water and vortexed for 2 min to release the fungal spores. 100 µl of the fungal spore solutions was dispensed on potato dextrose agar plates before being spread evenly with a sterile glass rod and allowed to stand for 1 h on the laboratory bench. Wells were then bored into the agar medium with a heat sterilized 6 mm cork borer. The wells were filled with 100 µl of different concentrations of the extract taking care not to allow spillage of the solution onto the surface of the agar.

The bacterial and fungal culture plates were allowed to stand on the laboratory bench for 30 min to allow proper diffusion of the extract and the antibiotics. The bacterial culture plates were incubated at 37℃ for 24 h while the fungal culture plates were incubated 27℃ for 3 to 7 days. Wells in blank Muell er Hinton agar and potato dextrose agar plates containing 10% acetone representing the final acetone concentration in the test plates without the extract served as positive controls. After 24 h incubation period, antibacterial activities were determined by measuring the inhibition zones against the test organisms using a calibrated transparent meter rule while the antifungal activities were determined by measuring the inhibition zones after 3 to 7 days of incubation. Both antibacterial and antifungal assays were carried out in triplicates.

Macrobroth dilution for minimum inhibitory concentration (MIC)

The activity of the extract and minimum inhibitory concentration were determined by the macrobroth dilution methods (NCCLS, 1993). For antibacterial assay, different concentrations of the extract (0.0097 to 10) mg/ml and ciprofloxacin (0.0097 to 5 µg/ml), used as positive control, were separately prepared by serial dilutions in Mueller Hinton broth. The tubes were inoculated with 100 µl of each of the bacterial strains. Blank Mueller Hinton broth was used as negative control.

For the antifungal assay, different concentrations of the extract (0.156 to 40 mg/ml) were prepared in sabouraud dextrose broth by serial dilutions. Each broth concentration was inoculated with 100 µl of the prepared fungal spores' solution. Two control tubes were included: one with spores and broth but no plant extract, and one with broth and extract but no spores. The bacterial containing tubes were incubated aerobically at 37℃ for 24 h. The fung al containing tubes were incubated at 27℃ for 3 to 7 days. The fir st tube in the series with no visible growth after incubation period was taken as the MIC.

Determination of minimum bactericidal and fungicidal concentrations (MBC/MFC)

For the determination of the MBC and MFC, fresh nutrient agar and potato dextrose agar plates were inoculated with one loopful of culture taken from each of the first three broth cultures that showed no growth in the MIC determination tubes. While MBC assay plates were incubated for 24 h, MFC assay plates were incubated for 3 to 7 days. After the incubation periods, the lowest concentration of the extract that did not produce any bacterial or fungal growth on the solid medium was regarded as MBC and MFC values for this extract (Irkin and Korukluoglu, 2007). This observation was matched with the MIC test tube that did not show evidence of growth after 48 h of incubating the bacteria or spore germination for the fungi after 3 to 7 days of incubation.

Determination of mechanisms of antibiosis (bactericidal or bacteriostatic)

The mechanism of antibiosis of the extracts was calculated using the ratio of MBC/MIC (MBC) or MIC_{index} as described by Shanmughapriya et al. (2008) to elucidate whether the observed antibacterial effects were bactericidal or bacteriostatic. When the ratio of MBC/MIC was ≤ 2.0, the extract was considered bactericidal or otherwise bacteriostatic. If the ratio is ≥16.0, the extract was considered ineffective.

RESULTS

The antimicrobial activity of the stem bark acetone extract of *E. caffra* was evaluated by measuring the zone of inhibition against medically important pathogens (Collins and Lyne, 1987). The degree of the antibacterial activity was assayed by serial two fold dilution method to determine the minimum inhibitory concentration (MIC) of the extract (Forrey et al., 1989). The result revealed that the acetone extract of *E. caffra* had good antibacterial activity against both Gram-positive and Gram-negative bacteria (Table 1) and antifungal activity against all the tested fungal isolates (Table 4). The antibacterial activity assessed in terms of inhibition zone indicated that *K. pneumoniae* ATCC 10031 had the highest inhibition zone while most of the bacteria had inhibition zones greater than 20 mm at the highest concentration of 20 mg/ml of the extract (Table 2).

The MIC values ranged between 0.0195 and 1.25 mg/ml while the minimum bactericidal concentrations (MBC) ranged between 0.0391 and 1.25 mg/ml for the extract. The minimum inhibitory concentrations assay indicated that the two groups of bacteria were susceptible to the extract at very high concentrations with *P. aeruginosa* ATCC 19582 having the highest minimum inhibitory concentration of 0.0195 µg/ml, thereby, showing the potential of the extract to inhibit Gram negative bacteria more than the Gram positive bacteria. In all cases, the MBC values were similar or 2 folds higher than MIC values for the extract. The MIC values of the crude acetone extract (0.0195 to 1.25 mg/ml) and the values of the inhibition zones were lower than the MIC values (0.0195 to 0.625 µg/ml) and inhibition zones obtained from ciprofloxacin antibiotics. The extract was less potent than the standard antibiotic, ciprofloxacin, to which the bacteria were highly susceptible at 5 µg/ml. The variation between the activities of the extract and the standard antimicrobial drug may be due to the mixtures of bioactive compounds present in the extract compared to the pure compound contained in the standard antibiotics (Gatsing et al., 2010). However, Wagner and Ulrich-Merzenich (2009) had earlier reported that antibiotics rarely have the same degree of activity as the unrefined extract at comparable concentrations or dose of the active component.

Table 1. Minimum inhibitory and bactericidal concentrations (MIC/MBC) of the acetone bark extract of *Erythrina caffra* Thunb.

Test bacterial strains	Bacterial susceptibility to Ciprofloxacin		Bacterial susceptibility to *E. caffra* extract		
	MIC (µg/ml)	MBC (µg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC_{index}
Streptococcus faecalis ATCC 29212	0.625	2.5000	0.6250	0.6250	1
Klebsiella pneumoniae ATCC 4352	0.1563	0.3125	0.3125	0.6250	2
Serratia mercescences ATCC 9986	0.0391	0.0781	0.6250	0.6250	1
Klebsiella pneumoniae ATCC 10031	0.0781	0.3125	0.0391	0.0781	2
Enterococcus cloacae ATCC 13047	0.1563	0.6250	0.6250	1.2500	2
Proteus vulgaris ATCC 6830	0.0391	0.0381	0.6250	0.6250	1
Shigella sonnei ATCC 29930	0.1563	0.1526	1.2500	1.2500	1
Escherichia coli ATCC 8739	0.1563	0.1563	1.2500	1.2500	1
Pseudomonas aeruginosa ATCC 19582	0.0781	0.1563	0.0195	0.0391	2
Klebsiella pneumonia KZN	0.0195	0.0391	0.6250	1.2500	2
Bacillus subtilis KZN	0.0781	0.0781	0.6250	0.6250	1
Shigella flexneri KZN	0.1563	0.1563	0.6250	0.6250	1
Staphylococcus aureus OK3	0.3125	0.6250	0.0781	0.1563	2
Staphylococcus aureus OK2a	0.6250	0.6250	0.6250	0.6250	1
Staphylococcus aureus OK1	0.0391	0.0391	1.2500	1.2500	1
Acinetobacter calcaoceuticus anitratis CSIR	0.3125	0.3125	0.3125	0.6250	2

The antifungal assay indicated that all the fungal isolates were highly susceptible to different concentrations of the extract and their susceptibilities were concentration dependent. With an increase in the concentration of the extract, there are increases in the inhibition zones of each of the fungal isolate (Table 3). The MIC and MFC values ranged between 0.625 and 20 mg/ml. While *C. glabrata* had an MIC value of 0.625 mg/ml and that of *A. flavus* was 20 mg/ml, the MIC values of *C. krusei*, *C. albicans*, *C. neoformans* and *A. corymbifera* was 2.5 mg/ml, *C. rugosa*, *T. mucoides* and *T. tonsurans* was 5 mg/ml, *P. notatum*, *A. terreus*, *A. niger* and *F. sporotrichioides* was 10 mg/ml. With the exception of *C. neoformans* and *A. corymbifera* having MFC which is 4 times greater than the MIC, the susceptibility of the other fungi to the extract indicated that the MFC values were 1 to 2 folds higher than the MIC values (Table 4).

DISCUSSION

Antimicrobial screening of traditional medicinal plants has been the source of innumerable therapeutic agents. While many complementary and alternative medicines have enjoyed increasing popularity recently, efforts to validate their use have seen their assumed effective therapeutic properties being increasingly scrutinized *in vitro* and *in vivo*. This is because a large number of antimicrobial agents derived from traditional medicinal plants are available for treating various diseases caused by microorganisms (Jain, 1994). Resulting from these scrutiny are evidences affirming that the therapeutical potential of plant based antimicrobial compounds without any side effects often associated with synthetic antimicrobials is inestimable. Consequently, many reports have indicated antimicrobial effects of medicinal plants against bacteria and fungi of medical importance. Though a number of antibiotics are available, increasing capability of microbes to develop multidrug resistance has further encouraged search for new, safe and effective bioactive agents of plant origin.

Approximating several antibacterial (Mathabe et al., 2006; Aiyegoro et al., 2008; Selowa et al., 2010) and antifungal activities (Dabur et al., 2007; Sharma et al., 2008; Sati and Joshi, 2011) of plants materials extracted with alcohols were mostly reported, this study indicates that acetone

Table 2. Inhibition zones produced by ciprofloxacin and extract against tested bacteria.

Test bacterial strains	Inhibition zones produced by ciprofloxacin (± 1.0 mm)			Inhibition zones produced by *E. caffra* (± 1.0 mm)		
	1.25 µg/ml	2.5 µg/ml	5 µg/ml	5 mg/ml	10 mg/ml	20 mg/ml
Streptococcus faecalis ATCC 29212	16	19	22	16	19	21
Klebsiella pneumoniae ATCC 4352	15	18	20	15	16	19
Serratia mercescences ATCC 9986	23	25	28	17	19	22
Klebsiella pneumoniae ATCC 10031	21	24	26	18	20	24
Enterococcus cloacae ATCC 13047	18	20	23	14	16	18
Proteus vulgaris ATCC 6830	23	26	32	18	20	22
Shigella sonnei ATCC 29930	18	20	23	16	18	20
Escherichia coli ATCC 8739	21	24	27	15	19	21
Pseudomona aeruginosa ATCC 19582	19	20	22	16	18	21
Klebsiella pneumoniae KZN	23	26	28	15	17	20
Bacillus subtilis KZN	24	27	30	16	18	21
Shigella flexneri KZN	15	18	20	15	17	19
Staphylococcus aureus OK3	17	20	22	17	20	22
Staphylococcus aureus OK2a	20	22	24	16	18	21
Staphylococcus aureus OK1	23	25	27	15	17	20
Acinetobacter calcaoceuticus CSIR	17	20	23	16	19	22

Table 3. Antifungal activities of different concentration of acetone extract of *E. caffra.*

Test fungal strains	Inhibition zones produced by *E. caffra*			
	5 mg/ml	10 mg/ml	20 mg/ml	40 mg/ml
Candida krusei	12	15	16	18
Candida albicans	0	10	10	12
Candida neoformans	0	10	12	13
Candida rugosa	0	11	11	12
Penicillium notatum	12	15	16	17
Aspergillus niger	12	13	14	15
Aspergillus terreus	11	12	13	14
Aspergillus flavus	0	10	12	14
Fusarium sporotrichioides	0	17	19	21
Absidia corymbifera	18	20	23	25
Candida glabrata	13	15	17	20
Trichophyton mucoides	0	15	18	20
Trichophyton tonsurans	0	16	19	22

Table 4. Minimum inhibitory and bactericidal concentrations (MIC/MBC) of the acetone bark extract of *E. caffra.*

Test fungal strains	Fungal susceptibility to *E. caffra*		
	MIC (mg/ml)	MFC (mg/ml)	MIC_{index}
Candida krusei	2.5	5	2
Candida albicans	2.5	5	2
Candida neoformans	2.5	10	4
Candida rugosa	5	10	2
Penicillium notatum	10	10	1
Aspergillus niger	10	20	2
Aspergillus terreus	10	20	2
Aspergillus flavus	20	20	1
Absidia corymbifera	2.5	10	4
Candida glabrata	0.625	0.625	1
Trichophyton mucoides	5	10	2
Trichophyton tonsurans	5	10	2
Fusarium sporotrichioides	10	20	2

extract could be as effective as other alcoholic extracts based on the degree of the attained antimicrobial activities of the extract. The extract inhibited the selected bacteria which are usually implicated as major culprits for human urinary tract infections, intestinal infections, food poisoning and nosocomial infections (Rossolini and Mantengoli, 2005; Olajuyigbe et al., 2006; Venier et al., 2007; Olajuyigbe and Adeoye, 2011) and the fungi causing major destruction and biochemical changes of food commodities, candidiasis and candida vaginitis, producing mycotoxins and carcinogens as well as serious threat to human health (Reddy et al., 2010) at low concentrations.

Although the MIC values of the extract against bacteria were considerably low, they were higher than those obtained for the fungi indicating that the bacteria were more susceptible to the extract than the fungi. The MIC/MBC and MIC/MFC ratios indicated that the extract is bacteriostatic and fungistatic at lower concentrations while at higher concentrations, significant bactericidal and fungicidal activities were exhibited against these potential pathogens. Where MIC equals MBC/MFC, the bactericidal and fungicidal potency of the components of the extract is affirmed. The antimicrobial activity of the extract, however, indicated a broad spectrum and great therapeutic potential of the plant. Although varied sensitivity may be attributed to different resistance levels between the strains (Cetin and Gurler, 1989; Ahmad and Aqil, 2007), the sensitivity inconsistency amongst Gram-positive and Gram-negative bacteria along with the fungal isolates could be ascribed to the anatomical or structural differences between these microorganisms (Olajuyigbe and Afolayan, 2011a).

The ability of this extract to inhibit *P. aeruginosa*, usually resistant to commonly used antimicrobial agents, is significant as this organism is a major cause of the morbidity and mortality in patients with chronic pulmonary infection (Govan and Harris, 1986) and a leading cause of progressive loss of lung function and early death (Pier, 2000). In harmony with earlier reports on the antimicrobial activity of some medicinal plant (Munoz-Mingarro et al., 2003; Coelho de Souza et al., 2004), *E. caffra* may offer new sources of antibacterial and antifungal agents with significant activity against infective microorganisms.

Although there is a dearth of sufficient scientific report on the pharmacological activities of *E. caffra,* the pharmacological properties of some members of *Erythrina* genus had been reported. Methanolic extracts of *E. senegalensis* (Saidu et al., 2000) and the acetone extracts of *E. abyssinica* (Yenesew et al., 2003) were reported to have anti-plasmodial activities due to the presence of flavonoids and isoflavonoids instead alkaloids. *E. mildbraaedii* has showed anti-oxidant and anti-inflammatory activities (Njamen et al., 2003). The extracts from *E. lysistemon* have also shown antiviral, anticancer and cytotoxic activities (El-Masry et al., 2002). The antimicrobial activity of this plant may be attributed to the presence of several secondary metabolites such as phenolics, alkaloids, flavonoids and tannins (Pandey et al., 2010; Olajuyigbe and Afolayan, 2011b) usually implicated in antimicrobial activities of medicinal plants.

In conclusion, the results indicate that the acetone extract of *E. caffra* exhibited a significant antimicrobial activity against bacteria and fungi suggesting the presence of either good antimicrobial potency or the high concentration of an active principle in the extract. The observed tendency of the extract to inhibit Gram-negative bacteria more than the Gram-positive and fungi at low concentrations showed that *E. caffra* contain interesting biopharmaceutical substances with ability to attract significant scientific attention.

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